ACUTE AND CHRONIC ASCORBIC ACID DEFICIENCIES IN THE RHESUS MONKEY [1]

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TWO FIGURES

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Signs of vitamin C deficiency in the Rhesus monkey (Macaca mulatta) have been described by many workers (Harden and Zilva, '18; Howe '23; Howitt, '31; Topping and Fraser, '39; Tomlinson, '39; Fraser and Topping, '42 and Tomlinson, '42). All these workers have emphasized the gingival changes which occurred.

Roff and Glazebrook ('39), Fitzsimmons ('41), Barahal and Priestman ('42), Kent ('43), Stuhl ('43) and Roth ('45) have described certain types of gingival changes in various groups of human beings which responded to ascorbic acid therapy. However, Crandon, Lund and Dill ('40) reported that in a case of acute scurvy experimentally produced in man, they observed no gingival changes. Pijoan and Lozner ('44), and Restarski and Pijoan ('44) believe that the assumption that gingivitis, with or without pyorrhea, is dependent upon a scorbutic basis is unwarranted unless there is antecedent or present clinical evidence of scurvy.

In our studies of ascorbic acid deficiency in the Rhesus monkey, very significant differences in the gross manifestations have been observed, depending on whether the deficiency was acute or chronic.

PROCEDURE

Ascorbic acid deficiency in the Rhesus monkey was produced on a ration composed of: sucrose 74%, acid-washed casein 18%, salts IV (Phillips and Hart, '35) 4%, and cottonseed oil 4%; 1:20 liver extract was added at a level of 3% at the expense of the entire ration. The daily

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vitamin supplement consisted of: thiamine 1 mg., riboflavin 1 mg., pyridoxine 1 mg., calcium pantothenate 3 mg., nicotinic acid 5 mg., choline 50 mg., inositol 100 mg., and para-aminobenzoic acid 100 mg. The supplement was given in 40–50 ml. of dilute sucrose solution which was placed in the water cup prior to feeding and watering. Once a week 0.5 ml. of halibut liver oil fortified to contain 500 I.U. of irradiated ergosterol was given orally to each monkey. Two control monkeys no. 129 and no. 130, were given the above ration and vitamin supplements plus 7.5 mg. of ascorbic acid per kilogram of body weight daily.

Four young monkeys (2 males, no. 100 and no. 102, and 2 females, no. 97 and no. 98) were used. Their weights ranged from 2.5 to 2.7 kg. and they were between 1½ and 2 years old. When an acute ascorbic acid deficiency appeared, a single dose of crystalline ascorbic acid, varying from 15 to 100 mg., was given to measure the ability of the acutely deficient monkey to respond to therapy. When the acute deficiency signs began to reappear, crystalline ascorbic acid was given daily at a level of 0.25 mg. per kilogram of body weight as a means of producing a chronic ascorbic acid deficiency. This subminimal level of ascorbic acid was continued for a period ranging from 80 to 100 days. When no. 100 was sacrificed for histological examination, the other three were given 7.5 mg. of ascorbic acid per kilogram of body weight, per day.

Later, two young male monkeys (no. 168 and no. 169) were used to study the complicating effects of biotin in the chronic stage of ascorbic acid deficiency. No. 168 received the same diet as those in the previous experiment, while no. 169 received in addition a daily supplement of 20 μg. of Merck's crystalline biotin. A second period of chronic ascorbic acid deficiency was produced in no. 98 during which 20 μg. of biotin were given orally.

Periodic examinations were made of the structures of the oral cavity. When a period of ascorbic acid deficiency began, bi-weekly examinations were necessary. At the end of these studies, the monkeys were sacrificed for histological examination.

RESULTS

The four monkeys which did not receive any ascorbic acid developed an acute deficiency in 30 to 50 days. The growth curves for these animals are shown in figure 1. The first sign of an imminent deficiency was the gradual loss of about 10% of the body weight over a period of approximately 2 weeks. In the next week, a more precipitous loss of an additional 10 to 15% of the original body weight occurred.

The coat of the acutely deficient monkeys became very rough without any loss of hair. Appetite decreased very rapidly during the period of weight loss. Even when the acute deficiency was allowed to become very severe, no gingival changes were observed in any of the four monkeys but the tooth surfaces were covered with a heavy residue.

One of the most profound changes observed, which paralleled the loss of weight in severity, was the apparent unwillingness of the monkeys to use their hind legs. This was first seen about a week after the precipitous weight loss began. The monkeys rarely left the bottom

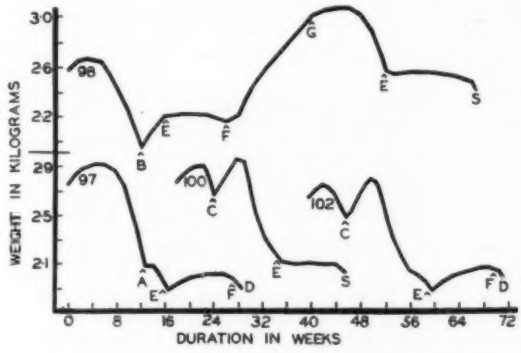

Fig. 1 Growth curves of four monkeys during period of acute and chronic ascorbic acid deficiencies.

A. 15 mg. of ascorbic acid given.
B. 30 mg. of ascorbic acid given.
C. 100 mg. of ascorbic acid given.
E. Began 0.25 mg. of ascorbic acid per kilogram of body weight per day.
F. Increased ascorbic acid to 7.5 mg. per kilogram of body weight per day.
G. Discontinued ascorbic acid therapy.
D. Died.
S. Sacrificed.

of the cage and locomotion consisted of the monkey pulling itself around by means of its arms. An acute tenderness of the joints, especially of the knees, was observed. When test doses of ascorbic acid were given, the appetite of the monkeys increased immediately and within a week the locomotor ability and the appearance of the hair coat was normal.

When the acute deficiency signs began to appear again, they were identical with those in the first period. Since the appetite of the animals decreased as the acute deficiency signs appeared and did not improve when 0.25 mg. of ascorbic acid per kilogram of body weight was given per day, 3 gm. of 1:20 liver extract were given to each monkey

daily by mouth. In this way the animals were supplied with these essential nutritional factors, normally supplied by the 1 : 20 liver extract contained in the ration.

The tenderness of the hind legs observed in the acute deficiency became rapidly worse in the chronic state. This was seen especially in no. 100 and no. 102. After 4 and 6 weeks, respectively, the soreness about the knees was extreme. Later in no. 100, a complete separation of the proximal end of the right tibia from the epiphysis was observed. The bony shaft of the tibia was located just under the skin in the region of the patella. In no. 97 and no. 98, the soreness became very great, but no separation was noted grossly.

Fig. 2 Late gingival changes in a chronic ascorbic acid deficiency. Hypertrophy and necrosis of gingiva and shifting of the deciduous molars.

For the first 10 to 14 days of the chronic deficiency, no changes were observed in the gingiva. Subsequently in all four monkeys, there was observed a swelling and hyperemia of the gums, especially in the interdental papillae. This condition became progressively worse with the hyperemia spreading to the buccal mucosa (fig. 2). The gums bled very easily and spontaneously as the chronic deficiency progressed. Pressure on the teeth, either in mastication or when applied during an oral examination, indicated a definite tenderness. When the spontaneous bleeding was most pronounced, there was necrosis of the gums, particularly of the interdental papillae. This necrosis spread until it involved large areas of the gums. Some alveolar resorption occurred which resulted in a loosening of the teeth and a premature loss of some of the deciduous molars.

When the gingival lesions began to appear, the white, sticky residue on the teeth observed in the acute deficiency became thicker, especially on the surfaces bordering the gums. As the gingival lesions progressed, these heavy deposits were replaced in some areas by thick regions of tartar.

Late in the chronic deficiency period, a severe loss of hair was observed over the entire body, particularly on the belly and hind legs. The skin was usually very dry with small regions of dermatitis on the face and belly. A mild edema occurred occasionally about the groin, upper legs and around the eyes. Hemorrhage occurred frequently about the eyes and more rarely on the body and extremities.

After 80 days of chronic deficiency, monkey no. 100 was sacrificed for histological examination. After 80 to 100 days of chronic deficiency, nos. 97, 98 and 102 were given 7.5 mg. of ascorbic acid per kilogram of body weight daily. Nos. 97 and 102 did not respond to ascorbic acid therapy but died within 2 weeks. No. 98 responded gradually; after a month of therapy, there had been a moderate increase in weight, a great increase in activity and a loss of soreness about the joints. However, there had been no improvement in the hair coat. At that time, 20 μg. of crystalline biotin were given daily. After 6 weeks, a new growth of hair began which developed until the monkey was completely covered.

The gingival lesions in no. 98 began to recede in the third week of therapy. The necrosis and bleeding were greatly reduced and finally disappeared. The gums never completely returned to their normal size and color but remained hypertrophic and hyperemic, especially in the regions where the most extensive necrosis had occurred and where deposits of dental tartar still remained. Sufficient necrosis had occurred in the lower right quadrant to cause a shifting of the deciduous and first permanent molars which persisted.

In the study of the effect of biotin upon the chronic ascorbic acid deficiency, the monkeys (nos. 98 and 169) which received 20 μg. of biotin per day maintained a normal amount of hair which became rough and unkempt. However, no. 168 exhibited a similar hair loss to the previous group of chronically deficient monkeys. The addition of biotin made no other difference in the signs of the chronic ascorbic acid deficiency.

Throughout these experiments, the control monkeys maintained a normal rate of growth comparable to those fed 3% 1:20 liver extract by Waisman et al. ('43). No gingival lesions, skin changes or hair loss were observed.

When the monkeys were sacrificed, post-mortem examinations were made. The skeletal changes were the most pronounced. In the six chron-

ically deficient monkeys, a very great increase in the size of the costochondral junctions was observed. In nos. 97, 100 and 102, there had been a complete separation of the proximal end of the tibia from the epiphysis. The proximal end of the tibia was very pointed and porous. The distal end of the femur was less affected, and no complete separation from the epiphysis was seen. Large areas of hemorrhage were observed between the epiphysis and the ends of the tibia and femur. Nos. 98, 168 and 169 showed similar but less extensive changes in the tibia and femur.

There had been considerable alveolar bone resorption in the areas of most extensive necrosis of the gingiva. This was most evident on the crest of the lateral plates. Extensive bone resorption was also observed over the exterior surface of the cranium and on both surfaces of the rami of the mandibles.

In monkeys nos. 97, 168 and 169 numerous carious lesions were observed in the first permanent molars. No lesions were observed in any of the deciduous teeth. In no. 97 there were 8 lesions: 3 in each upper molar and one in each lower molar. Thirteen lesions were observed in no. 168: 4 in each upper molar, and 2 and 3, respectively, in the left and right lower molars. Eight lesions were observed in no. 169: 4 and 2, respectively, in the left and right upper molars, and one in each lower molar. No carious lesions were observed in the other chronically ascorbic acid deficient monkeys, nor in the two control monkeys.

DISCUSSION

Two distinct syndromes of ascorbic acid deficiency have been observed in the Rhesus monkey depending on whether the deficiency was acute or chronic. An acute deficiency was characterized by a precipitous weight loss and tenderness in the joints of the legs but no gingival lesions. A chronic deficiency was characterized by severe gingival lesions and skeletal changes but no rapid decrease in weight.

The signs of a chronic ascorbic acid deficiency are very similar to those described by the workers who produced an ascorbic acid deficiency in monkeys by the use of natural rations (Harden and Zilva, '18; Howe, '23; Topping and Fraser, '39; Fraser and Topping, '42 and Tomlinson, '42). The gingival lesions are especially similar to those described in scorbutic monkeys by Tomlinson ('39), by Fraser and Topping ('42) and by Tomlinson ('42) and in scurvy in man by Hess ('20).

Roff and Glazebrook ('39), Fitzsimmons ('41, '42), Barahal and Priestman ('42), Kent ('43), Stuhl ('43) and Roth ('45) have described gingival lesions in man which receded when ascorbic acid was

given. These authors stated that there were other cases of periodontal disease which did not respond to ascorbic acid therapy. The gingival lesions which did respond to therapy appeared to be similar to the gingival lesions described in classical scurvy in man (Hess, '20) and in chronic ascorbic acid deficiency in the Rhesus monkey.

Recently Crandon, Lund and Dill ('40) and Restarski and Pijoan ('44) have described cases of experimental scurvy in man, in which they observed no gross changes in the gums or teeth. Pijoan and Lozner ('44) and Restarski and Pijoan ('44) believe that the assumption that gingivitis rests on a scorbutic basis is unwarranted unless there are accompanying clinical evidences of late scurvy. These experimental cases of scurvy in man are similar to the acute deficiency in the monkey where no gingival lesions were observed.

Since there are such great differences in the manifestations of the acute and chronic phases of an ascorbic acid deficiency in the monkey, it is necessary to consider these differences when scurvy in man, experimental or spontaneous, is being studied and described.

Howe ('23, '27) reported a high incidence of carious lesions in scorbutic monkeys which were fed a natural diet. We have observed caries in three of six chronically ascorbic acid deficient monkeys but none in the two controls. The number of animals is too small to consider the caries incidence significant. However, the frequency of carious lesions in young monkeys which have been on experiment for only 8 months is usually so low that a 50% incidence in this small group and the high number of lesions per animal may indicate that the caries index is increased in a chronic ascorbic acid deficiency.

SUMMARY AND CONCLUSIONS

An acute ascorbic acid deficiency was produced in young Rhesus monkeys in 30 to 50 days. In the acute phase there was a precipitous loss in weight, slight tenderness of the joints and heavy residues on the tooth surfaces. However, no gingival changes were observed. When a single test dose of ascorbic acid was given, there was a very rapid and complete alleviation of these signs.

In the chronic state of ascorbic acid deficiency, a series of gingival changes was observed which was accompanied by increasing food deposits on the teeth and dental tartar. There was very marked tenderness and soreness at all the joints accompanied by a considerable swelling. Bone resorption occurred at the bone-cartilage junctions, especially at the ends of the long bones of the legs. There was a very extensive loss of hair and a mild dermatitis; both could be prevented or

cured by crystalline biotin. In three of the six chronically deficient monkeys, there was a high incidence of dental caries.

These data indicate that the signs of acute and chronic ascorbic acid deficiencies in the monkey are distinctly different. They may partially explain the differences observed in experimentally produced and spontaneous scurvy in man.

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THE AVAILABILITY OF VITAMINS FROM YEASTS

I. THE ABSORPTION OF THIAMINE BY HUMAN SUBJECTS FROM VARIOUS TYPES OF BAKERS' YEAST [1]

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It was shown as early as 1933 by Walker and Nelson that rats were not able to utilize thiamine from fresh yeast so well as that from dried yeast containing fewer viable cells. This was confirmed by Parsons and Collord ('42). Parsons, Collord, Strong and Peterson ('42) and Parsons and Collord ('42) found that boiled yeast was a better source of thiamine for human subjects than fresh unboiled yeast. It was shown that thiamine not accounted for by the urinary excretion during the periods of fresh yeast ingestion was largely present in the feces so that the failure of the urinary thiamine to rise was attributable to lack of absorption.

Opportunity was afforded for studying simultaneously with human subjects and with rats the absorption of thiamine from several types of bakers' yeast; these proved to represent three levels of thiamine concentration, as well as some diversity in regard to the content of other vitamins. The results of the human studies are presented in this paper;[4] supporting data on the same yeasts when fed to rats are given elsewhere (Parsons et al., '45).

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Certain of the results have appeared in abstract form in the Proc. of the Fed. of Am. Soc. for Exp. Biol. for 1944, vol. 3, p. 96. This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] Some of the experimental data have been taken from a thesis submitted by Miss Williamson in partial fulfillment of the requirements for the degree of Master of Science in Home Economics. Present address: Department of Nutrition, School of Public Health, Harvard University.

[3] Research fellow in Home Economics, summer 1944, under a grant from the Pineapple Research Institute of Hawaii and now in the Laboratory of Nutrition Research, North State Teachers College, Denton, Texas. E. Price and C. Plisch carried out some of the thiamine assays under the Pineapple grant also.

[4] Generously supplied by Eli Lilly Co.

EXPERIMENTAL

Determinations are reported of the urinary thiamine eliminations of twenty-three human subjects (fecal, of eleven) on controlled basal diets to which were added, in test periods of 3 to 5 days, samples of compressed bakers' yeasts typical of those found on the market (see table 1 of following paper by Parsons, Foeste and Gilberg, '45). Yeasts A and C were of different strains and contained 0.5 mg. of thiamine per cake. In a recently introduced modification of yeast A, designated here as A_2, the thiamine content was doubled, although this change was in no way evident from the label. Yeasts C, D, D_2 and E were made from the same batches as were corresponding samples of yeast F but were modified by the manufacturers not only by increases in their thiamine content (30-fold for yeast C and 250- to 300-fold for D, D_2 and E) but also in certain other respects. Their manufacture will be further discussed below. Yeasts A and A_2 were procured for the experiment on the open market. The other samples were furnished by the manufacturers in weekly shipments. The extent to which the various yeasts would release thiamine available for absorption during digestion was tested by feeding them both fresh (as purchased) and after boiling. The method of preparing the heated yeast was as follows: The fresh yeast was broken up in a small amount of water and the suspension was poured into rapidly boiling water which was maintained at the boiling point for 10 to 120 seconds and consumed after cooling. The uncooked yeast dose was suspended in an equal quantity of cold water. In the case of yeast A, the uncooked yeast dose was also consumed, in some instances, suspended in tomato juice (diet 1) or pineapple juice (diet 2) or eaten as the solid cake. Such variations in these and in some additional experiments (Link) had no effect on the usual low output of urinary thiamine or the appearance of live yeast cells in the feces. In control periods, thiamine hydrochloride [5] was substituted for the yeast sources in equivalent amounts.

Two basal diets were employed: basal diet 1, an ordinary type of diet similar to that described by Parsons and Collord ('42), and basal diet 2, a dairy products-pineapple-bread diet (Parsons, Stettler, Williamson and Johnson, '44). Further details of the experiment are presented in the tables. In all cases, the basal diet supplied sufficient thiamine to meet the metabolic requirements of the subjects and, hence, urinary excretions were considered to reflect the absorption of thiamine furnished by supplements. However, as a special precaution some subjects were given thiamine hydrochloride previous to the experiment (table 3). The subjects comprised graduate or senior women of the

[5] Generously supplied by Eli Lilly Co.

University of Wisconsin and were judged to be in a satisfactory nutritional state.

Subjects of study 1 participated in two test diet periods separated by an interval of 60 days, during which yeast D was ingested in the fresh form. The plan of this experiment originated in the suggestion of other workers that the prolonged ingestion of fresh yeast would enable the body to become so habituated to it that the availability of the thiamine would be improved. Yeast D was chosen for this test inadvertently on the assumption that thiamine was as poorly absorbed from this type as it had been from the other yeasts investigated. Only after the 60-day interval of fresh yeast ingestion was in progress was it realized from the results of urine assays from the previous period that yeast D, in the fresh form, supplied available thiamine; hence, instead of a test for habituation to raw yeast, the interval served to load body stores with thiamine to the extent that urinary excretions for all subjects were significantly higher during the second test period (table 1).

TABLE 1

Thiamine intake and average daily urinary and fecal thiamine elimination of subjects consuming yeast D both fresh and after treatment by boiling.

STUDY 1 [1] (3 SUBJECTS)						STUDY 3 [1] (1 SUBJECT)				
Period	Thiamine intake		Thiamine output			Period	Thiamine intake		Thiamine output	
	Basal [2]	Supplement	Urinary excretion		Fecal output		Basal [2]	Supplement	Urinary excretion	Fecal output
			test I	test II	test I					
	mg./day	*mg./day*	*µg./day*	*µg./day*	*µg./day*		*mg./day*	*mg./day*	*µg./day*	*µg./day*
Basal (4 days)	2.0	..	440	520	500	Basal (5 days)	1.0	..	315	115
Boiled yeast (4 days)	2.0	3.7	930	1335	800	Fresh yeast (5 days)	1.0	2.0	560	605
						Basal (3 days)	1.0	..	285	160
Fresh yeast (4 days)	2.0	3.7	1000	1420	880	Boiled yeast (5 days)	1.0	2.0	625	645
Thiamine hydrochloride (4 days)	2.0	3.7	....	1750	...	Thiamine hydrochloride (5 days)	1.0	2.0	610	420

[1] Biological assay of thiamine, study 1; chemical assay, study 3.

[2] Basal diet 1 to which was added 1.0 mg. crystalline thiamine hydrochloride per day throughout the four periods.

[3] Basal diet 2 including 600 gm. canned pineapple juice per day.

One subject of study 3 (table 2) consumed, for 3 days, yeast A treated by freezing; when it became apparent from the assays that the absorption of thiamine from this form of yeast was not improved over that from the fresh form, the yeast was fed after boiling as a control test for 1 day; even to this brief period the subject responded promptly with increased urinary thiamine excretion.

TABLE 2

Thiamine intake and average daily urinary and fecal thiamine eliminations of subjects of groups 2 and 3 consuming yeasts A, C or E both fresh and after treatment by boiling.

Period	STUDY 3								STUDY 2			
	Thiamine intake		Average daily thiamine output						Length period	Thiamine intake		In urine yeast A (3 subj.)
			Yeast C (3 subj.)		Yeast E (3 subj.)		Yeast A (3 subj.)					
	Basal [1]	Supplement	Urine	Feces	Urine	Feces	Urine	Feces		Basal [2]	Supplement	
	mg./day	*mg./day*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*days*	*mg./day*	*mg./day*	*µg./day*
Basal (5 days)	1.0	..	200	225	210	260	155	450	3	1	.	130
Fresh yeast (5 days)	1.0	2.0	185	1115	295	350	100 [3]	2625	3	1	1	80
Basal (3 days)	1.0	..	190	740	200	150	150	220	.	.	.	..
Boiled yeast (5 days)	1.0	2.0	670	315	670	160	525	...	3	1	1	330
Thiamine hydrochloride (5 days)	1.0	2.0	880	485	880	130	885	610	3	1	1	290

[1] Basal diet 2 including 600 gm. canned pineapple juice.

[2] Basal diet 1.

[3] One subject of this group in a 3-day period consumed the fresh yeast after it had been frozen for 24 hours. See discussion of results.

All thiamine determinations were made by the thiochrome method, essentially that of Hennessey ('42). Duplicate determinations were made on separate days for individual samples. Periodic recoveries of standard amounts of thiamine added to the samples averaged 95% and were for the most part within the range 92–96%. Food aliquots representing 10% of the weighed daily food consumption were homogenized and assayed in the fresh form for thiamine.

RESULTS AND DISCUSSION

Two yeast samples with comparable thiamine content, A and C, gave results in agreement with earlier data on yeast A and B (Parsons and Collord, '42). Apparently, these yeasts contributed little or no thiamine to the body when fed to human subjects in the amounts ordinarily recommended (yeasts A and C, table 2; yeast C, table 3). It even appears that thiamine from the basal diet was taken up to an appreciable extent by these types of fresh yeast and so bound that it became largely unabsorbable in the digestive tract. This was indicated by the consistently diminished urinary thiamine excretion during the fresh yeast period as contrasted to the preceding basal period whether the amount of yeast ingested was two or four cakes. The average decrease in the urinary thiamine output during the period of fresh yeast feeding in comparison with the preceding basal period was, for the six subjects on yeast A, 37%; for the six subjects on yeast C, 17%. This effect of fresh yeast seemed to carry over slightly into the succeeding short basal period for those subjects for whom this period was provided. Although alternative explanations for the lessened urinary thiamine in the fresh yeast period might well be offered, the fecal thiamine values tend to substantiate the hypothesis stated above.

Thiamine from yeast D, in contrast to that from yeasts A and C, was nearly as available from the fresh as from the boiled yeast during duplicate tests in study 1 and also in a later test with another subject (study 3, table 1). That this difference was valid and fundamental was confirmed by the results of animal assays (described in the following paper) which gave uniformly high values for the utilization of fresh yeast D for all assays during the 15-month interval between the two human experiments above. Unexpectedly, however, the human and rat tests for the extent of absorption of thiamine from this fresh yeast simultaneously showed a lower assay value than formerly in comparison with boiled yeast D or with equivalent thiamine hydrochloride. So unmistakable was the change that this new form of the yeast was designated yeast E (see table 2) and information on possible alterations in its production was sought from the manufacturers. It was discovered that, beginning with the date on which the change in assay value occurred, yeast D had been taken off of the market; the sample which was sent to this laboratory was made in small amounts as a special courtesy and only thiamine hydrochloride added instead of other substances as well (see table 1 of following paper). However, in a new sample, the manufacturers scrupulously reproduced every step of the former procedure for yeast D, as far as possible. This sample (yeast

D_2) fed fresh yielded very nearly the same assay values as had yeast D (table 3). The procedure in the tests with yeast D_2 was such that the results removed the possibility that the difference in the urinary outputs on yeasts D and E might have been due to the change in sequence of periods or the total intake of thiamine or of yeast. The strain of yeast was not an essential factor in explaining the difference between

TABLE 3

Thiamine intake and average daily urinary output for subjects consuming yeast A_2, C or D_2.

Period	STUDY 4				STUDY 5					
	Thiamine intake		Thiamine in urine		Thiamine intake		Thiamine in urine			
							Yeast D_2		Yeast C	
	Basal [1, 2]	Supplement	Yeast D_2 2 subj.	Yeast A_2 2 subj.	Basal [1]	Supplement	1 subj.[3]	1 subj.	1 subj.[3]	2 subj.
	mg./day	*mg./day*	*avg. µg.*	*avg. µg.*	*mg./day*	*mg./day*	*avg. µg.*	*avg. µg.*	*avg. µg.*	*avg. µg.*
Basal (4 days)	2	.	565	590	1	.	215	290	310	295
Fresh yeast (4 days)	2	2	1080	850	1	2	465	610	180	245
Basal (3 days)	.	.	...	...	1	.	275	435	215	...
Boiled yeast (4 days)	2	2	1380	1505	1	2	550	...	550	...
Thiamine hydrochloride (4 days)	.	.	...	...	1	2	655	...	655	...

[1] Basal diet 2 including 600 gm. whole crushed canned pineapple per day. Small, fixed increases in the thiamine intake of individual subjects resulted from slight dietary modifications necessitated by controlling protein intake.

[2] 1.0 mg. of thiamine hydrochloride daily was added to the basal diet of group 4 throughout the three periods.

[3] Given 3.0 mg. of thiamine hydrochloride for 4 days before the experiment; 2 days elapsed without the dose before the first basal period.

E and D_2, inasmuch as yeast C, from which D_2 was made, lowered the urinary thiamine when fed fresh as it had in the earlier tests (tables 2 and 3). Laxation produced by the various yeasts was ruled out as a decisive factor causing the differences in absorption of the thiamine from them by results recently published elsewhere (Parsons, Stettler, Williamson and Johnson, '44; Stettler, '44).

In the meantime, during a routine re-assay of the thiamine of yeast A it was discovered that the manufacturers had modified this yeast by doubling the thiamine content as well as by the changes declared on the label (table 1 of the following paper). Hence, this new yeast, designated as yeast A_2, was fed to two members of a diet squad (study 4, table 3). It did not behave as yeast A had in former repeated trials with respect to decreasing the output of urinary thiamine in comparison with a preceding basal period, although, as in the case of E, the absorbability of its thiamine was still much improved by boiling the yeast. The relative urinary increase of thiamine due to the intake of fresh yeast A_2 was somewhere between that of yeast E which had a higher thiamine content and yeast D which had, in addition, a higher content of other factors. This would be in harmony with an hypothesis that various kinds and concentrations of substances added to the yeast cell may influence the readiness with which the cell gives up its thiamine in the digestive tract. Some specially prepared commercial yeast samples are being studied at present in an investigation of this theory.

It is evident that subjects receiving D, D_2 and E as the thiamine supplements were required to take much smaller amounts of yeast to supply the desired thiamine intake than were subjects ingesting the types with a lower thiamine content. It might reasonably be postulated, therefore, that this smaller amount of yeast would be more vulnerable to the digestive processes and, hence, offer less difficulty in releasing its thiamine high up in the digestive tract. That the influence of the amount of yeast was negligible, however, within the limits of the experiment and that other factors played a more important role is evident from the data. For example, the feeding of yeast A, fresh, in the amounts of either 2 or 4 cakes (about 25 or 50 gm.) unfailingly resulted in reducing the urinary output of thiamine in comparison to that on the same diet without it. Fifty grams of fresh yeast A_2, however, increased measurably the urinary output of thiamine instead of decreasing it. As little as 8 gm. per day of yeast E was only fairly well utilized in contrast to 8–18 gm. of yeast D and D_2 from which the thiamine was apparently more readily absorbed.

The generalization in a preliminary report from Melnick ('44) that only about 16% of the thiamine in fresh yeast is available to human subjects cannot be discussed in relation to the present results until fuller publication is available and the identity of the type of yeast in Melnick's experiment is known.

Preliminary experiments in this laboratory on the fecal elimination of live yeast cells have been confirmed and extended. Consistently

negative results were obtained when the subjects consumed boiled yeast or no yeast, but positive results whenever fresh yeast was consumed. Assays of total 24-hour collections of fecal material for periods of several days following the ingestion of 50 gm. of fresh yeast showed a prompt elimination of a considerable number of living yeast cells and the persistence of a diminishing number of 1 or more days following this. Carmine was ingested to mark the beginning of the fecal period, and the dose of yeast was consumed several hours later. Following are the total daily values obtained for two subjects expressed as the number of thousand viable yeast cells eliminated on consecutive days: For subject M.L.J., yeast A, 565000, 182 and none; yeast D_2 none, 5122, 19, none and none; for subject C.M., yeast A, 177 and 46; yeast D_2, 15, 148000, 110, 31 and 64. As the highest elimination represented only between 1 and 2% of the total number of yeast cells consumed, it is, perhaps, not surprising that no quantitative relationship was seen between viable yeast cells and the relative urinary excretion of thiamine on the two yeasts, the latter possibly being dependent on rapid absorbability of thiamine at a fairly restricted segment of the digestive tract while destruction of yeast cells presumably persisted far beyond such an area.

In agreement with former results, it has again been found true that the amounts of thiamine in urine and feces on certain fresh and boiled yeasts show a somewhat reciprocal relationship (yeasts A and C, table 2). It appears that the failure of the urinary thiamine level to rise in response to the ingestion of thiamine in the form of fresh yeasts A and C was chiefly due to lack of absorption of the vitamin from the intestinal tract, inasmuch as the fecal thiamine content was significantly lower when thiamine hydrochloride or these yeasts in the boiled form were fed, than when the yeasts were fed fresh. It may be noted that one subject (table 2, study 3, yeast A) eliminated 2.6 mg. of thiamine per day in the feces during the fresh yeast period. Although this amount was usually high, somewhat comparable amounts (1.9 mg. avg. per day) had been reported previously with the same type of yeast (Parsons and Collord, '42).

CONCLUSIONS

1. Two types of bakers' compressed yeast, fed fresh to human subjects were poorly utilized as sources of thiamine as indicated by low urinary and high fecal eliminations of thiamine. The ingestion of these yeasts appears even to have decreased the amount of available thiamine supplied by the basal diets, inasmuch as the urinary thiamine was reduced below that of the preceding basal period and fecal thiamine

was much increased. Living yeast cells were recovered from the feces after fresh yeast feeding.

2. Marked improvement in thiamine absorption from these yeasts was obtained by boiling them for brief periods.

3. Four fresh yeast samples, manufactured by the same two firms that prepared the first two types described but with higher vitamin content, did not show the previously observed effect of lowering the urinary output of thiamine in human subjects but led to varying degrees of increased thiamine excretion instead; the yeast most notable in this regard was the one with the highest vitamin levels. The explanation for this apparent correlation has not been determined.

4. The differences in the absorbability of the thiamine from various fresh yeasts did not seem to be attributable either to the strain of yeast or to the amount of yeast consumed by the subjects.

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THE AVAILABILITY OF VITAMINS FROM YEASTS

II. THE ACCESSIBILITY TO RATS FOR GROWTH OF THE THIAMINE IN VARIOUS TYPES OF BAKERS' YEAST [1]

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ONE FIGURE

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The availability to human subjects of thiamine from yeasts just reported (Parsons, Williamson and Johnson, '45) was judged on the somewhat indirect although acceptable evidence of the presence of additional thiamine in the urine following doses of yeast and a corresponding absence of a striking increase in the feces. The indication thus obtained of differences in the absorbability of the thiamine of various yeasts has been strengthened by similar observations of differences in utilization obtained by the more direct evidence of variations in the body weight of rats on given doses in biological assays carried out simultaneously, to be reported in this paper.

EXPERIMENTAL

The various compressed bakers' yeasts studied in these experiments are identified in table 1. All doses of yeast were administered separately instead of being incorporated in the basal ration. This was particularly desirable in view of the pronounced change in odor and taste of the yeasts under various treatments which, in itself, might otherwise have influenced the intake of the ration. A high palatability of the basal diet was also sought. The percentage composition of the ration selected, adapted from one used by Schlutz and Knott ('36, '39) was: purified casein 15; fresh pig liver autoclaved at 120° for 5 hours and dried 15; sucrose 48.5; Crisco 17; and salt mixture (Osborne and Mendel, '19) 4.5. All rats received a vitamin supplement containing the following per rat per week: riboflavin 200 μg.; choline 35 mg.; nicotinic acid 170 μg.;

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by Purnell funds. Reported in part in a preliminary abstract (Parsons, Foeste, Williamson and Stettler, '44).

[2] Present address: Dietitian with the armed forces in France.

calcium pantothenate 1.5 mg.; pyridoxine 200 μg.; and 6 drops of cod liver oil. This supplement was distributed in several doses per week or, in the instance of pyridoxine, was incorporated in the basal ration for some rats.

The preparation of yeast doses for feeding consisted in suspending a weighed portion in water and making it up to a suitable volume. The individual doses of yeast and vitamin supplements were fed directly to the rat by pipette except when the eagerness of the rat for the dose made this difficult to manage, in which case the measured dose was offered in a small feeding dish and the rat was watched for the few moments necessary for the quantitative ingestion of the dose.

In preparing the liver, an autoclave was used which allowed the reading of temperatures directly rather than by pressure gauge, inasmuch as it was believed by Knott [3] that precision in heat treatment is necessary for a reliable degree of destruction of thiamine without too great injury to other nutritive factors. Assurance of the satisfactory absence of thiamine from the basal ration was given by the regularity and swiftness of the depletion of the negative control rats, i.e., a sharp cessation of growth at the end of 7 to 12 days during which there was an average total gain of 34.6 gm.; and death after typical convulsions on an average of 32 days (range, 28 to 36 days) and an average total weight loss of 41.8 gm. A dose of 4 μg. of thiamine hydrochloride [4] per day produced an average gain of 1.5 gm. body weight for the positive control rats of 150 gm. or less. Beyond this weight, the dosage was raised to 5 and 6 μg. for rats up to 250 gm. body weight, for about the same rate of gain. The level of intake of basal ration of these rats on such levels of thiamine supported apparent vigor and the absence of abnormalities during the 16 to 32 weeks of the experiment except for some brown scurf under the thick hair along the back and a barely detectable amount of reddish stain on the hair around the nose and on the forepaws.

In conformity with the Schlutz and Knott's ('36) method, a short feeding period (10 to 15 days) was adopted for several reasons. For example, although the composition of the basal ration was presumably unfavorable to extensive intestinal synthesis, the short period was thought to be an added precaution against any tendency for fresh yeast, fed constantly over long periods, to become established in the rats' digestive tract or to encourage some special type of bacteria, which might conceivably obscure the assay through synthesis of thia-

[3] Personal communication.

[4] Generously supplied by Eli Lilly Company.

mine. Human subjects in this laboratory showed living yeast cells in the feces for several days after a single dose of fresh yeast (Parsons, Williamson and Johnson, '45). In actual tests, however, a somewhat contrary tendency was frequently observed in the rats held for several weeks on fresh yeast doses at high levels which seemed at first to be quite adequate, namely, an unaccountably sharp and extensive decline after vigorous growth. This type of curve was not observed on doses of thiamine hydrochloride or boiled yeast and, hence, was difficult to evaluate in the assay, making long periods and high dosage of doubtful assay value. In addition, the short period was well adapted to the detection of possible changes in the potency of the manufactured yeasts, since speedy identification of any such changes with even slight alterations in procedure in the factory might yield valuable data.

A uniform dosage was used in both of the alternating periods of fresh and treated yeast samples. This was done not only because of the difficulty, just mentioned, with the higher doses but also for the purpose of avoiding diverse levels of intake of other factors from so potent a material as yeast. Even though a level of dosage satisfactory in the case of treated yeast often led to losses of body weight when fresh yeast was fed, nevertheless, the satisfactory gains in weight in the following period on treated yeast appeared to justify the procedure.

The three treatments of the yeast used were selected from the standpoint of their effect on the viability of the yeast cells. One treatment consisted in suspending yeast in about 5 parts of 80% ethyl alcohol for 24 hours, then reducing it to dryness before a fan at room temperature. Other samples were suspended in 3 parts of water and brought rapidly to boiling which was maintained for 10 to 120 seconds.

Prolonged periods of freezing such as are known to kill yeast cells had been found in another laboratory [5] to be effective in increasing the utilization of the thiamine of yeast for rats. Inasmuch as lengthy periods would be impractical as a household means of rendering the thiamine of yeast more available for absorption, shorter periods were tested. Cakes of yeast in their individual wrappers were put into the freezing unit of a household electric refrigerator at — 5°C. and left for 24 hours. In an attempt to increase the disruption of the yeast cell during freezing and thawing, other samples were suspended in 5 times their weight of liquid. A nutrient solution was used, inasmuch as this was being employed in other experiments on yeast at the time. It contained 1.0 gm. di-ammonium sulfate and 50.0 gm. anhydrous dextrose

[5] Personal communication from Prof. N. B. Guerrant, Department of Biological Chemistry, Pennsylvania State College.

per liter. The doses of frozen yeast were fed either immediately after thawing or 1 hour later. In plate counts carried out subsequently, it was established that none of these procedures was significantly injurious to the yeast cell.

RESULTS

The wide range of variation shown in the availability of thiamine of different yeasts consumed fresh by human subjects (Parsons, Williamson and Johnson, '45) was noted also when these same yeasts were fed to rats. As in the human experiments, here also there were only two yeast samples, i.e., those with the highest vitamin content, yeasts D and D_2, which appeared to be about equally effective sources of thiamine whether fed fresh or after boiling or treatment with alcohol; the availability of the thiamine of the other six samples fed was much lower and was significantly increased by these means. One of these six samples, F, was not fed to human subjects in our experiments because of the limiting low thiamine content (table 1) but from its position in the series with respect to C, D and E, its low thiamine availability and the improvement in this due to boiling would be expected.

With the particular technic used in these experiments for comparing the accessibility of thiamine from fresh as contrasted with treated yeasts in the digestive tracts of rats, minor variations between yeasts such as that between yeasts A and A_2 were not clearly demonstrated as they were in the human experiments. This seems reasonable when it is considered that the basal diet of the rat was essentially devoid of thiamine, whereas, much of the minor variation apparent in the effect of the various fresh uncooked yeasts on the urinary output of thiamine of human subjects appeared to depend on the extent of influence of the fresh yeast on the thiamine of the ingested food. Experiments aimed at reproducing the dietary conditions of the human experiments more closely in the rat are under way in this laboratory.

Boiling the poorly utilized fresh yeast samples and soaking them in alcohol were equally effective in improving the availability of the thiamine as seen in comparable tests in different groups of rats and in alternate periods of the same rats (fig. 1, groups C 2 and C 3). This similarity of effect of the two processes might be expected from the equal destruction of viability of the cell which they entailed. The short periods of freezing, on the other hand, were practically non-injurious to the cell, and, correspondingly, no measurable improvement in the growth of the rats was seen on frozen yeast A as compared with that of unfrozen

TABLE 1

Characterization of the baking yeasts tested for the availability of their thiamine.

IDENTITY OF THE YEASTS [1,2]	MANUFACTURER	TYPE YEAST	MANUFACTURERS' DECLARATION OF POTENCY [3,5]	WEIGHT OF YEAST WHICH FURNISHED 1 MG. THIAMINE
				gm.
A	1	Consumers' foil	Two cakes supply 100% of the adult minimum daily requirements for A, D, B_1, and 14 to 20% for B_2 plus 2 to 3 mg. niacin	25.2
A_2	1	Consumers' foil	Two cakes supply 100% of the adult minimum daily requirements for A, B_1, B_2, D plus 10 mg. niacin	12.6 [4]
B	2	Consumers' foil	12.0 mg. B_1 per pound 6.0 mg. B_2 per pound	37.8
C	2	Special [5]	12.0 mg. B_1 per pound 6.0 mg. B_2 per pound	37.8
D	2	Enriched [5]	90 to 114 mg. B_1 per pound 6.0 mg. B_2 per pound 800 mg. niacin per pound 600 mg. iron per pound	5.1 4.0
D_2	2	Enriched [5]	114 mg. B_1 per pound 6.0 mg. B_2 per pound 800 mg. niacin per pound 600 mg. iron per pound	4.0
E	2	High thiamine [5]	114 mg. B_1 per pound 6.0 mg. B_2 per pound	4.0
F	2	Regular [5]	3.3 to 3.6 mg. B_1 per pound 50 to 55 mg. niacin per pound 15 to 20 mg. iron per pound 6.0 mg. B_2 per pound	137.5

[1] Yeast B was fed to human subjects in a previous experiment (Parsons and Collord, '42). Yeast F required so high an intake to supply 1–2 mg. of thiamine for human subjects that it was tested only with rats. The other six yeast samples were fed from identical lots to the human diet squads and the rats reported in these two papers.

[2] Yeast samples C, D, D_2 and E were all made from the same batches as were corresponding samples of yeast F but were modified by the manufacturers.

[3] Thiamine was added as the hydrochloride to yeasts B, C, D, D_2 and E by the manufacturer. It was not stated in what form thiamine was added to yeasts A and A_2. All the types of yeast contained a small amount of some form of starch and a trace of oil.

[4] The thiamine concentration in yeast A_2 was found by biological and chemical assay in this laboratory to be double that in yeast A, although it could not be so interperted from the label.

[5] For use by bakers.

fresh yeast (fig. 1, groups A 4 and A 5). This again was in agreement with the human tests. These results with freezing and those of the Pennsylvania Laboratory are in agreement in that the utilization of the thiamine was correlated with the viability of the yeasts in both cases.

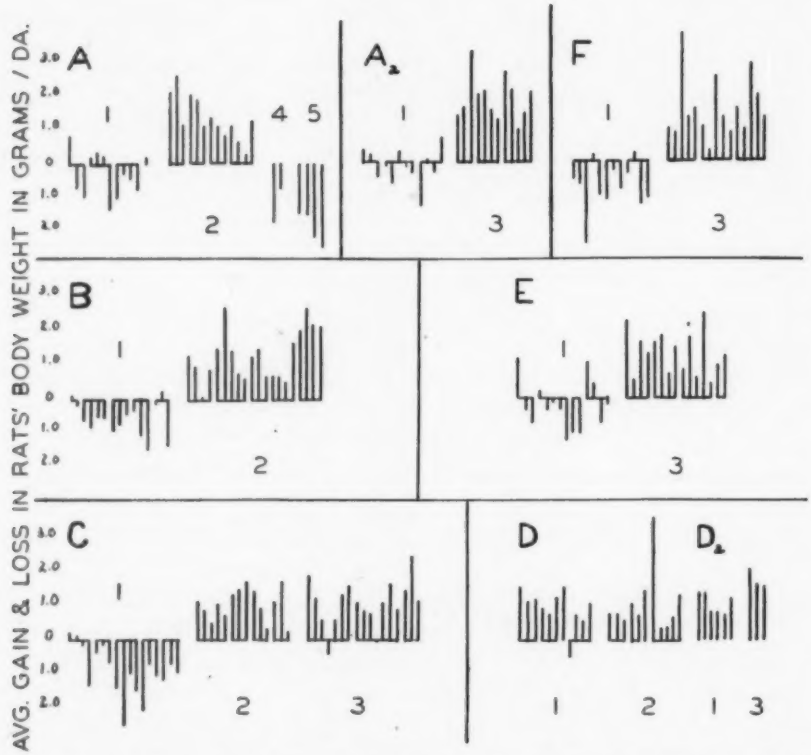

Fig. 1 Lines represent the average daily gain or loss in grams of body weight of rats on fresh and treated yeasts, actually fed in alternating periods but the records regrouped arbitrarily for comparison.

Letters refer to the brand or sample of yeast; numbers, to the treatment as follows: 1, fresh, raw yeast; 2, treated with alcohol; 3, boiled; 4, frozen in cake; 5, frozen in liquid.

Each group of lines banded together represents the repeated (although not consecutive) performances of one rat on one given yeast preparation. For example, the first line in A 1 indicates that the rat (after an initial 9-day depletion period on the basal ration) gained 0.9 gm. per day on fresh, raw yeast A and in the succeeding 10-day period on boiled yeast, gained 2.2 gm. per day (first group under A 2). Then in alternating fresh and boiled yeast periods, the rat lost 0.7 gm.; gained 2.7 gm.; lost 1.0 gm.; and gained 1.3 gm. (on the average daily). Only a part of the total assays carried out are presented but these were taken in blocks from consecutive rats' records and, hence, were unselected.

CONCLUSIONS

Biological assays of the thiamine of fresh yeasts showed that this was relatively unavailable to rats in six of the eight yeast samples tested but was rendered available for growth when the fresh yeasts were boiled or treated with alcohol.

The two samples of yeast which were nearly as effective sources of thiamine for growth when the yeast was fed fresh, as purchased, as after boiling or treatment with alcohol, were the two with the highest vitamin content of the series fed.

Short periods (24 hours) of freezing had no measurable effect in increasing the availability of thiamine from a poorly utilized fresh yeast.

These results of rat assays are in general agreement with the tests on human subjects on these same yeast samples and preparations from them.

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THE RETENTION OF THIAMINE, RIBOFLAVIN AND NIACIN IN COOKING PORK AND IN PROCESSING BACON

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The question of the retention of the B vitamins in meat during cooking and other processing is of primary importance in assessing the contribution of these vitamins to the diet by this important food group. Waisman and Elvehjem ('41) have published a compilation of the available data on the vitamin content of meat to that date. The data are largely concerned with raw meat and there is little information on the losses in cooking. Since that time several papers concerning the losses to be expected in cooking meat under specified conditions have been published. A large part of this work has been done at the University of Wisconsin (McIntire, Schweigert, Henderson and Elvehjem ('43); Schweigert, McIntire and Elvehjem ('43); and McIntire, Schweigert and Elvehjem ('43)). Additional information has been contributed by Cover, McLaren and Pearson ('44) and by Lane, Johnson and Williams ('42). Despite this work it was felt that there was a need for further data by other laboratories to provide a more complete picture. The effect of variations in the cooking procedure on the retention of the vitamins has not been fully investigated, other workers having used only optimal conditions. Exact cooking procedures are seldom used in the average household and the effect of common errors in cooking on the retention of vitamins must be understood to arrive at a figure for retention representative of less exact procedures. Cover, McLaren and Pearson ('44) gave this matter some consideration when they reported the retentions in rare and well done beef, but no results of this nature have been reported for pork.

This paper is the report of an investigation of the amount of thiamine, riboflavin and niacin in various pork cuts, the variation to be expected between carcasses and between cuts from the same carcass,

[1] Under the direction of Alan Brown, M. D., F.R.C.P. (Lond.).

the retention of these vitamins in roasting and frying under optimal conditions, and the effect of variations from these optimal conditions. There is included some information on the retention of these vitamins in the processing of bacon.

METHODS OF ASSAY

The samples were of a size that would be handled ordinarily in domestic use. The entire sample was ground finely in an electric meat grinder and thoroughly mixed by hand. A 100-gm. sample of this mixture was then weighed out and homogenized with 400 ml. of 0.1 N HCl in a Waring blendor. This blended sample was kept in the refrigerator for assay. Tests showed that the sample could be preserved in this way for at least 6 weeks with no detectable loss of thiamine, niacin or riboflavin. Samples of this meat-acid mixture were weighed out for assay of the individual vitamins.

Thiamine was assayed by the thiochrome method (Hennessy, '41; Hennessy and Cerecedo, '39). Some difficulty was encountered in preparing a suitable extract containing all the thiamine in the meat. Lane, Johnson and Williams ('42) have reported similar difficulties. At first a simple extraction with dilute acid at 100°C. followed by digestion with takadiastase at pH 4.5 was tried. When this extraction procedure was used to test the keeping properties of thiamine in the meat-acid mixture, it was found that there was a gradual increase in the apparent thiamine. This increase was more marked if the meat-acid mixture was preserved at room temperature than when it was kept in the refrigerator. This observation pointed to the presence of some complex of thiamine not extractable in the free form by this procedure but gradually decomposed on standing. Various enzymes were tried in addition to the takadiastase to effect this decomposition, and the following extraction procedure was evolved. A sample of the meat-acid mixture was weighed out in a small beaker. One hundred mg. of pepsin per gram of meat was dissolved in water to make a 5% solution and added to the meat-acid sample. This was mixed and incubated for 4 hours at 37°C. At the end of this time the sample was mixed with 50 ml. of 0.1 N HCl and the mixture steamed for 1 hour in an Arnold steamer, cooled, and the pH adjusted to about 4.5, using 2.5 M sodium acetate solution. Two hundred mg. of takadiastase and 200 mg. of maltase, dissolved together in 10 ml. of water, were added to the mixture and incubated over night at 37°C. The solution was then diluted to 100 ml. and filtered. Higher values for thiamine were obtained with this procedure and there was no evidence of liberation of further

amounts from the meat-acid mixture on standing. Recovery of thiamine added to the meat-acid mixture ranged from about 90 to 100% for a series of tests. The average recovery was 94.5%. The thiamine content of the various enzyme preparations was so small that it was neglected without introducing an appreciable error.

Riboflavin was assayed by the microbiological assay method of Snell and Strong ('39). Extracts were prepared by autoclaving with 0.1 N HCl, neutralizing to pH 5.0 to 5.5, and filtering cold. There was a voluminous precipitate at this pH which effectively entrapped any fatty material. The precipitate carried down a small amount of riboflavin, approximately 10% of the total. Accordingly, the precipitate was re-extracted by autoclaving with 0.1 N HCl, neutralizing and filtering. It was washed several times with water and the combined extracts and washings adjusted to pH 6.8, diluted to an appropriate volume, and filtered again. The filtrates were crystal clear and there was no evidence of interference by fatty materials. No further riboflavin could be found in the precipitate. Loss of riboflavin in standard solutions heated under the same conditions of temperature, time and acidity was only about 2%.

A preliminary comparison of the chemical and microbiological assay procedures for niacin was made before the selection of an assay method for this constituent. The chemical assay method used was that of Dann and Handler ('41), with a few minor modifications. The hydrolysis with hydrochloric acid was modified by heating at 15 pounds steam pressure for ½ hour instead of heating on the water bath for 1 hour. The adsorption on Lloyd's reagent, elution, and clarification with lead nitrate was not changed. The development of the color was modified to use half the amounts of clarified extract and reagents, maintaining the same relative concentrations. The heating period with CNBr, was 10 minutes exactly at 75°–80°C., the temperature not being allowed to fluctuate outside this maximum range. The 5% metol reagent was made up in 5% acetic acid. The addition of the acetic acid resulted in an augmented color production. The color was read on an Evelyn photoelectric-colorimeter, using Filter 400. Recovery of added niacin ranged from 90 to 104% in ten experiments, and averaged 94.2%.

The microbiological assay was that of Snell and Wright ('41) as modified by Krehl, Strong and Elvehjem ('43). Several extraction procedures were tested. The first of these consistd simply of extraction in 0.1 N hydrochloric acid by autoclaving at 15 pounds for 1 hour. The mixture was cooled, neutralized to pH 6.8, diluted to volume and filtered. The second procedure used the same acid extraction. The mix-

ture was then cooled and made approximately 0.1 N with respect to NaOH by addition of 10 N NaOH. It was then heated to boiling and allowed to cool to room temperature. The alkali was neutralized to pH 6.8 with HCl and the extract diluted to volume. This procedure had previously been used with cereals to hydrolyze the alkali labile niacin complex present in that type of material. The third procedure consisted of heating on a boiling water bath for ½ hour in 4% NaOH. The alkali was then neutralized to pH 6.8 with hydrochloride acid, the mixture diluted to volume and filtered. A portion of the clarified filtrate used for the color development step of the chemical assay was appropriately diluted and assayed by the microbiological procedure.

TABLE 1

Comparison of microbiological and chemical assays for niacin in meat — (μg./gm.).

ASSAY METHOD	EXTRACTION METHOD	BEEF				PORK			
		Raw		Cooked		Raw		Cooked	
		Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Microbiological	0.1 N acid extraction	53.5	62.5	99.0	81.0	34.3	34.0	51.0	46.3
	0.1 N acid extraction then hydrolyzed in 0.1 N NaOH	52.2		101.0		35.1		53.0	
	4% NaOH extraction	52.7		101.0		34.2		52.0	
	Acid hydrolysis and clarification as for chemical assay	57.0	62.2	96.1	77.0	34.9	34.6	50.5	50.2
Chemical		62.5	65.0	101.0	82.8	40.5	39.4	56.3	54.6

Table 1 shows comparative results by these methods on some beef and pork samples, both raw and cooked. The cooked samples appearing in this table were made by heating the ground raw meat in a covered cast iron frying pan until all the liberated juices had been concentrated to the point that they could be absorbed again on the meat. The meat was then reground and thoroughly mixed. It was found that all the extraction procedures gave comparable results with the microbiological assay, well within the experimental error of the assay method. However, the chemical procedure gave results that were higher than those obtained by the microbiological method, with the exception of one

sample of cooked beef. The discrepancy was more marked with the pork than the beef samples. The fact that the microbiological assays on the same clarified extracts used for the chemical assays were lower than the chemical assays and comparable with the microbiological assay results obtained on other extracts indicates that the higher values obtained with the chemical method were not due to liberation of extra niacin by the more vigorous treatment used in the chemical procedure for preparation of extracts. The agreement between the microbiological assays on the clarified extracts and the other extracts is good evidence of the specificity of the biological response, since it is probable that any foreign materials that might influence the biological response given by the relatively crude extracts prepared by the first three methods, would be eliminated by the treatment with Lloyd's reagent and precipitation with lead nitrate used for the preparation of the clarified extract. Furthermore, the agreement between microbiological assays by all four extraction procedures indicates that all the procedures probably extract the niacin completely.

The microbiological assay method was selected for use in this investigation, the 0.1 normal acid extraction procedure being used. However, eleven raw pork samples and six cooked pork samples were assayed by both the chemical and microbiological procedures. It was found that the chemical procedure was higher by an average of 45% on the fresh pork and 25% on the cooked pork.

The B vitamins in raw pork

Information was first obtained on the variations in the B vitamins that could be expected between various samples of raw pork. This was required in order that some estimate could be made of the significance of the variations in the vitamin content of the cooked meat samples and their raw controls.

The variation between cuts from a single carcass was determined by dividing a carcass into its commercial cuts. Each cut was boned and the meat was ground and mixed. The variations observed between the different cuts, with the exception of the tenderloin, were not marked. The mean variation of the thiamine was $\pm$ 9%, that of the riboflavin was $\pm$ 4%, while the mean variation of the niacin was $\pm$ 9%. However, the tenderloin contained a much higher content of all the B vitamins than any other cut. The thiamine, riboflavin and niacin content of the tenderloin was 57%, 50% and 45%, respectively, higher than the mean of the same vitamin for the rest of the carcass.

Table 2 shows the variations for picnic hams and butts between various carcasses and between opposite sides of the same carcass. The meat cuts used for analysis were commercially trimmed, i.e., the skin was removed and some of the fat trimmed. The weight of the picnic hams averaged 3100 gm., while that of the butts averaged 3700 gm. The series was too small to warrant an extensive statistical analysis; however, the following calculations were made. The average variation of the thiamine content of the individual hams from the mean thiamine value for all the hams was approximately ± 19%. Similarly the average variation for the butts was ± 36%. The same variation of the riboflavin content was ± 6% for the hams and ± 4% for the butts,

TABLE 2

Variations between carcasses and between sides of the same carcass. (μg./gm. fresh meat basis).

CUT	CARCASS NO.	RIGHT SIDE				LEFT SIDE			
		Fat	Thiamine	Riboflavin	Niacin	Fat	Thiamine	Riboflavin	Niacin
		%				%			
Picnic ham	2	25.7	9.83	1.28	38.9	24.1	7.04	1.25	43.2
	3	25.3	7.30	1.25	42.0	31.0	8.11	1.19	31.6
	4	24.3	. . .	1.07	42.6	24.1	8.93	1.25	33.0
	5	16.5	6.53	1.21	44.5	25.5	6.4	1.22	26.8
Butt	6	27.9	5.3	1.14	29.5	28.3	5.45	1.16	33.0
	7	32.3	10.7	1.17	44.2	24.3	10.2	1.24	41.5
	8	27.3	6.9	1.25	35.6	33.3	6.2	1.25	34.6
	9	29.7	7.2	1.22	33.9	24.9	7.5	1.26	36.3
	10	30.2	5.3	1.22	29.0	29.0	5.3	1.24	27.4

while the variation of the niacin content was ± 15% for the hams and ± 20% for the butts. The variation of the right and left cuts of a carcass from the mean value for the carcass was also calculated, and the mean value of these variations for all the carcasses was determined. This value for the thiamine in picnic hams was found to be ± 7%, and was ± 2% for the butts. The value for this variation for riboflavin was ± 3% in the hams and ± 1.5% in the butts, while that for the niacin was ± 14% in the hams and ± 3% in the butts. It can thus be seen that the variations between carcasses were much more than the variations between the sides of a single carcass. Calculation of the assay results on a fat-free basis did not decrease the variations of the thiamine and niacin values and considerably increased that of riboflavin. Therefore, all later results were calculated on the basis of the whole sample and no attempt was made to correct for variations in the fat content of the samples.

The B vitamins in cooked pork

The small variations noted between butts from the two sides of a single carcass led to the selection of this cut for the sample used in the experimental determination of the losses of the B vitamins in the roasting of pork. The butts were divided in halves to limit further the variations and to obtain roasts of a size commonly used domestically (about 1800 gm.). The halves were numbered 1 and 2, the numbers referring to anatomically the same half in each case. No. 1 half-butt from one side and no. 2 half-butt from the other side of a carcass were then roasted while the remaining halves were reserved as raw controls. The six butts from three carcasses were used for the series for each

TABLE 3

The retention of B vitamins in roast pork.

		TIME OF COOKING IN MIN. PER LB.	SHRINK.	THIAMINE			RIBOFLAVIN			NIACIN		
				Raw	Cooked	Retention	Raw	Cooked	Retention	Raw	Cooked	Retention
			%	μg./gm.	μg./gm.	%	μg./gm.	μg./gm.	%	μg./gm.	μg./gm.	%
Correctly cooked	Max.	45.0	43.0	6.78	5.98	68.0	1.30	2.12	113.0	26.5	38.0	88.5
	Min.	35.0	30.0	5.35	3.58	38.0	1.12	1.46	84.5	23.5	23.4	65.0
	Avg.	40.0	38.5	6.36	4.75	46.0	1.18	1.82	96.0	25.1	31.0	78.0
Underdone	Max.	35.0	44.0	7.78	7.15	74.5	1.34	2.19	123.0	29.0	38.0	121.0
	Min.	19.0	25.6	3.09	2.13	35.5	1.15	1.91	90.0	12.6	19.3	72.0
	Avg.	26.3	36.3	5.54	5.08	57.5	1.25	2.06	105.0	20.8	30.3	94.0
Overdone	Max.	40.5	50.0	6.83	4.34	45.0	1.20	2.20	107.0	29.2	41.0	99.0
	Min.	33.0	34.0	3.35	2.10	28.2	1.13	1.73	80.0	15.5	19.3	70.0
	Avg.	36.7	43.7	5.19	3.42	36.8	1.18	1.93	92.0	20.7	29.0	75.2

type of roasting. Mean retention values for each series can thus be considered significant to within ± 2% for thiamine, ± 1.5% for riboflavin, and ± 3% for niacin, on the basis of the small series in table 2. Actually a figure of about 5% for all the vitamins would probably be better, as the series in table 2 was too small to warrant a close interpretation of the statistical analysis.

The first cooking experiments were designed to determine the losses due to roasting pork. A digest of the results of these experiments is given in table 3. Three methods of cooking were investigated. The half-butts were boned and rolled before cooking in all cases. The first method, labelled "correctly cooked" in the table, consisted of roasting the pork in an open pan in an oven at 350°F. until the internal tem-

perature at the center of the roast reached 185°F. The next cooking method, labelled "underdone," was designed to illustrate the results of attempting to hurry the cooking by using a hotter oven. For experimental purposes the meat was roasted in an open pan at 450°F. until the exterior was nicely browned. The internal temperature of the roast at this time was 150°F.–155°F. and the interior was distinctly underdone. The third method of cooking, labelled "overdone," comprised roasting in an open pan at 400°F. until the internal temperature reached 190°F. By this time the exterior of the roast was just beginning to char. Roasting was done in the oven of an ordinary domestic electric stove. Oven temperature was controlled by a thermostat. The accuracy of the temperature setting of the thermostat was checked with a standard mercury thermometer. The roasts were weighed before and after cooking and the loss in weight was calculated as the per cent shrinkage. The individual roasts were then ground and mixed for sampling. The mean values and the maximum and minimum values are reported in the table. The vitamin content is expressed as micrograms per gram of the ground sample as weighed out. The retention was found by calculating the vitamin content of the cooked samples on a raw basis by multiplying by $\frac{100 - \%\ \text{shrink}}{100}$ and comparing this figure with the vitamin content of the corresponding raw control. The necessity of determining the dry weights of all samples and calculating the results on a dry weight basis was thus eliminated.

The mean retentions of the three B vitamins that were investigated were found to be highest in the underdone roasts and lowest in the overdone roasts. There was little or no loss of riboflavin. The mean retentions varied from 92% in the overdone meat to 105% in the underdone meat. The loss of niacin was also quite small. However, about half the thiamine was lost, the mean retentions ranging from 36.8% in the overdone roasts to 57.5% in the underdone roasts.

The retention of the B vitamins in frying pork chops was investigated next. Samples for frying and for raw controls were selected by the following procedure. An entire pork loin was sliced into chops about ¾-inch thick. As the chops were cut off they were placed successively in one of three piles. The first chop was placed in pile no. 1, the second in pile no. 2, the third in pile no. 3, the fourth in pile no. 1, the fifth in pile no. 2, the sixth in pile no. 3, and so on until the chops from the entire loin had been divided among the three piles. One pile was ground and mixed to serve as the raw control for the other two piles from the same loin. These piles were fried, ground and sampled separately. There were about eight individual chops in each pile.

The ranges and mean values of the data obtained from the experiments on frying chops are given in table 4. The effect of overcooking and undercooking was again investigated. The cooking method designated as "correctly cooked" was recommended by Canada Packers, Ltd., and was found to produce a well cooked juicy product. The frying pan was heated until it just began to smoke. The chops were browned on one side and the grease was then discarded. The chops were then browned on the other side and cooking completed by heating in the open pan in the oven at 325°F. for 15 minutes. The undercooked chops were simply browned rapidly in a hot pan. The total cooking time was about 10 minutes and the interiors of the chops were underdone

TABLE 4

The retention of B vitamins in fried pork chops.

		SHRINK.	THIAMINE			RIBOFLAVIN			NIACIN		
			Raw	Cooked	Retention	Raw	Cooked	Retention	Raw	Cooked	Retention
		%	μg./gm.	μg./gm.	%	μg./gm.	μg./gm.	%	μg./gm.	μg./gm.	%
Correctly cooked	Max.	46.0	8.24	8.06	58.5	1.45	2.12	81.0	21.0	38.7	103.0
	Min.	34.5	5.15	4.69	53.0	1.46	1.72	72.5	23.9	32.9	88.0
	Avg.	39.0	6.70	6.25	56.8	1.46	1.84	76.9	22.5	35.5	96.6
Underdone	Max.	32.5	8.68	9.49	87.5	1.49	2.17	103.0	23.1	32.4	123.0
	Min.	29.5	5.83	6.49	73.5	1.33	1.63	82.5	18.2	30.5	93.0
	Avg.	31.2	7.26	8.16	78.0	1.41	1.87	91.1	20.7	31.8	107.0
Overdone	Max.	48.0	6.20	5.64	52.0	1.38	2.05	78.0	24.2	46.0	111.0
	Min.	44.5	5.09	4.86	42.0	1.46	1.77	64.5	21.6	30.9	68.0
	Avg.	46.6	5.65	5.12	48.8	1.42	1.92	75.1	22.9	38.6	91.0

but not raw. The overdone chops were browned rapidly on both sides in a hot pan and then covered and cooked on the top of the stove for about 20 minutes. These chops were considerably shrunken and dried.

Here again, as in the case of roast pork, the less cooking the meat received the greater the retention of the vitamins. The retention of thiamine ranged from a mean of 78% in the underdone chops to 48.8% in the overdone chops. The extent of this variation in retention was somewhat greater than that found for roast pork. The retention of riboflavin ranged from a mean of 91.1% to a mean of 75.1%, while that of niacin ranged from 107% to 91%. It is interesting to note that the retention of thiamine and niacin is greater in fried chops than in roast pork receiving a similar degree of cooking, while the retention of riboflavin is lower.

The B vitamins in bacon

The results of an experiment designed to determine the retention of the B vitamins following the curing and smoking of bacon are reported in table 5. The experiment was undertaken in cooperation with Canada Packers, Ltd., and the curing and smoking methods used were their standard processes. The "wet cure" processing of sides used for the production of "Devon" brand bacon and the "dry" or "box" cure processing of sides and backs used for the production of "Maple Leaf" brand bacon, were investigated.

TABLE 5

The retention of the B vitamins in processed bacon.

PROCESS		THIAMINE			RIBOFLAVIN			NIACIN		
		Fresh	Processed	Retention	Fresh	Processed	Retention	Fresh	Processed	Retention
		µg./gm.	µg./gm.	%	µg./gm.	µg./gm.	%	µg./gm.	µg./gm.	%
Wet cure (Devon) side	Max.	6.14	4.59	84.0	1.30	1.12	102.0	34.8	25.6	90.5
	Min.	3.40	2.48	67.0	0.99	1.09	79.0	18.3	16.6	68.0
	Avg.	4.41	3.53	74.1	1.15	1.10	89.0	25.4	21.9	80.8
Dry cure (Maple leaf) side	Max.	4.66	3.99	96.0	1.34	0.91	63.5	24.3	23.8	115.0
	Min.	2.43	2.16	75.5	1.22	0.68	45.5	16.8	21.4	78.5
	Avg.	3.68	3.40	83.0	1.29	0.80	55.4	20.8	22.3	97.0
Dry cure (Maple leaf) back	Max.	11.5	8.07	109.0	1.72	1.13	62.0	39.5	47.3	109.0
	Min.	5.83	6.66	62.5	1.61	0.96	52.0	32.3	31.2	86.0
	Avg.	8.18	7.37	85.0	1.66	1.08	57.9	37.0	40.1	95.9

Retention calculated on 89% yield of bacon for the dry cure for both side and back, and 92% yield for the wet cure side. These figures are for average yields as supplied by Canada Packers, Ltd.

Four pieces of pork were selected for testing each process. Two of these pieces were selected from the shoulder end of the full sides or backs, and the remaining two were chosen from the ham ends. Each piece was about 18 inches long. About 5 to 6 inches of each piece was cut off and ground raw to serve as the control. The remaining piece was carried through the process and ground for the processed sample. The rind was removed in all cases.

The "wet cure" gave the lowest retention of thiamine, with an average of 74.1%. The "dry cure" gave 83% retention for the sides and 85% retention for the backs. The retentions of riboflavin show the greatest difference between the "wet" and "dry" cures. The retention by the "wet" cure was 89%, while the "dry" cure retained only

55.4% and 57.9% in the sides and backs, respectively. The retention of niacin was best by the "dry" cure, being nearly 100% for both the side and back bacon, whereas 80.8% was retained by the "wet" cure. Hence somewhat better retention of thiamine and niacin is obtained by the "dry" cure processing of bacon, but the "wet" cure process retains a much larger percentage of the riboflavin.

DISCUSSION

The raw samples of pork assayed throughout the study showed the following content of the B vitamins. The thiamine content of eight loin samples averaged 6.95 μg./gm., with a maximum range from 5.15 μg./gm. to 9.06 μg./gm. The riboflavin content of the same samples averaged 1.36 μg./gm. and ranged from 1.09 to 1.49 μg./gm., while the niacin content averaged 26.3 μg./gm. and ranged from 18.2 to 42.6 μg./gm. Twenty-nine samples of raw pork butts showed an average thiamine content of 6.22 μg./gm., with a range of 3.09 to 10.7 μg./gm. The riboflavin content averaged 1.21 μg./gm., with a range of 1.12 to 1.34 μg./gm., and the niacin content averaged 30.8 μg./gm., with a range of 12.6 to 44.2 μg./gm. The loins appeared to be somewhat higher than the butts in thiamine and riboflavin and slightly lower in niacin, but the ranges overlapped considerably for all three vitamins.

McIntire, Schweigert, Henderson and Elvehjem ('43) report the assays on fresh loin and fresh ham control samples. Their assays for thiamine ranged from 7.7 to 14.8 μg./gm. for hams, and 7.4 to 15.2 μg./gm. for loins. Riboflavin ranged from 2.1 to 3.4 μg./gm. for hams, and 1.7 to 3.0 for loins. Niacin ranged from 31 to 38 μg./gm. for hams, and 31 to 49 for loins. These ranges for thiamine and riboflavin are distinctly higher than those found by us, the riboflavin values, particularly, being approximately twice as high. The range reported for niacin falls in the upper limits of the range we have found. The higher content of thiamine and riboflavin found in raw pork by the Wisconsin group may be the result of differences in feeding methods used for growing and fattening hogs in the United States and Canada. Miller, Pence, Dutcher, Ziegler and McCarty ('43) report an increase of 100% in the thiamine content of pork muscle when the thiamine intake was increased from 1.318 mg. to 3.447 mg./pound of feed.

Comparison of the retention figures reported by McIntire et al. ('43) with those we have found reveal a further difference. They report an average retention of 68% of the thiamine in roast pork, while we found a retention of 46% for pork that had been correctly cooked by our methods. Even pork that was obviously undercooked retained only

57.5% of the thiamine. However, the retentions of riboflavin and of niacin agree fairly well with the retention of 90% of the riboflavin and 79% of the niacin reported by these workers.

The retention of thiamine in fried pork chops was found to be higher than that in roast pork butts. This observation parallels that made by Schweigert, McIntire and Elvehjem ('43) on roasted and fried ham. These workers also observed a higher retention of niacin by frying, although the difference they noticed was not as large as we observed. However, we observed a lowered retention of riboflavin by frying, which was not found in the work on ham. It must be realized that this work on cured ham is not strictly comparable to our work on fresh pork. The difference between our observations and those of the Wisconsin group on the retention of thiamine in roast pork point to the desirability of further study on this important subject.

CONCLUSIONS

The variation between cuts from a single carcass (with the exception of the tenderloin) and between the right and left sides of a carcass is much less than between different carcasses.

The more the pork is cooked, whether it is roasted or fried, the lower is the retention of thiamine, riboflavin, or niacin.

The greatest loss occurs in thiamine in either roasting or frying, the losses of riboflavin or niacin being comparatively small.

Frying gives a higher retention of thiamine than roasting.

The "wet cure" method of processing bacon gives a lower retention of thiamine and niacin than the "dry" or "box cure" but gives a much higher retention of riboflavin.

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DENTAL CARIES IN THE COTTON RAT [1]

III. EFFECT OF DIFFERENT DIETARY CARBOHYDRATES ON THE INCIDENCE AND EXTENT OF DENTAL CARIES

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Shaw et al. ('44, '44 a) have reported a high incidence and extent of carious lesions in the molars of cotton rats fed purified rations high in sucrose. In direct contrast, a low incidence and extent of caries were observed in those cotton rats fed natural rations or purified rations where the sucrose was replaced by coarse or fine dextrin. Since such a definite difference was obtained by the replacement of the sucrose by dextrin, an investigation was carried out to determine the effect of several different carbohydrates in purified rations on the incidence and extent of carious lesions. The results of these studies and the growth data obtained from these experiments and those previously reported on dental caries are presented in this paper.

EXPERIMENTAL

The cotton rats used were obtained from our stock colony. Those on the carbohydrate experiments were weaned at a weight of 15–20 gm. rather than at a weight of 20–25 gm. as was done in the previous work. This change was made since it appeared that the animals were able to adapt themselves more readily to the experimental conditions at the earlier age. As nearly as possible males and females were equally divided within each experimental group. Similarly, members of each litter were distributed equally between all groups in any experiment. After the animals had been on experiment for 14 weeks, they were sacrificed for the observation of the incidence and extent of tooth de-

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cay. The techniques of observation and evaluation of the carious lesions were the same as described in the earlier work (Shaw et al., '44, '44 a).

The percentage composition of the sucrose basal ration 801 is as follows: sucrose 73, casein 18, salts IV 4, corn oil 5; and adequate quantities of the B vitamins (McIntire, Schweigert and Elvehjem, '44) were provided. Each rat was given one drop of halibut liver oil per week. Ration 802 was prepared by replacing 6% of the sucrose in ration 801 with casein, which brought the protein level to 24%.

The other carbohydrate diets were prepared in a similar manner by replacing the sucrose with either maltose,[2] glucose,[3] lactose,[4] dextrimaltose [5] (both with and without added NaCl), fructose,[6] fine dextrin, coarse dextrin or soluble starch.[7] The water of crystallization of glucose (cerelose) and maltose was taken into account in the preparation of these rations. The addition of 4% of 1:20 liver extract had been shown previously to increase the growth rate (McIntire et al., '44), and not to alter the incidence or extent of dental caries (Shaw et al., '44). Therefore 4% of 1:20 liver extract was added, at the expense of the entire ration, to all the rations used in the carbohydrate experiments.

TABLE 1

Comparison of the rate of growth of males and females over a 6-week period.

RATION	MALES		FEMALES		PER CENT DIFFERENCE A-B/B × 100
	No. of animals	Grams gain per wk. (A)	No. of animals	Grams gain per wk. (B)	
802	14	6.9	18	5.6	23
802 + 4% 1:20 L.E.	20	9.0	11	7.2	25

In table 1 a summary of the data on the growth rate of males and females obtained from thirty-two rats fed the sucrose basal ration 802 and from thirty-one animals fed ration 802 + 4% 1:20 liver extract is presented. It is obvious that the males consistently grew at a more rapid rate. In order to express the growth data as one figure for each group the values for the females were increased by 25%. Since only a limited number of animals were available for experiments, it was not possible to use animals of one sex in any single experiment.

[2] C.P. hydrate, Pfanstiehl Chemical Company.
[3] Cerelose, Corn Products Refining Company.
[4] U.S.P., Merck and Company.
[5] Mead Johnson and Company.
[6] d-Levulose C.P. special, Pfanstiehl Chemical Company.
[7] Reagent grade, Pfanstiehl Chemical Company.

Various fractionations of 1:20 liver extract were made and the fractions were fed to cotton rats which received rations 801 and 802. All the liver fractions were added at the expense of the entire ration. Data on the rate of growth are included for groups of animals from earlier dental caries studies in order to compare the growth results with the severity of tooth decay. The average gains per week for both the first 6 weeks and 14 weeks on experiment are presented in table 2. The incidence and extent of carious lesions obtained when the different carbohydrate diets were fed are shown in table 3.

RESULTS

When rations 801 and 802 were fed, the average rate of growth in the first 6 weeks was approximately the same, namely 6.7 and 6.9 gm. per week, respectively. The rate of growth was more uniform when ration 802 was fed; therefore, 24% casein was used in all rations subsequent to this observation. When whole liver substance, 1:20 liver extract or the alcohol extract of 1:20 liver extract were added to ration 801, the growth rate was increased to 10.3, 8.1, and 7.7 gm. per week, respectively. Similarly solubilized liver, 1:20 liver extract, or the alcohol extract of 1:20 liver extract increased the growth rate when added to ration 802, to 8.2, 9.0, and 7.8 gm. per week, respectively.

The growth rate and ability of the cotton rat to survive were markedly affected by the particle size of the complex carbohydrates. When fine dextrin, soluble starch or fine stock rations were ingested, inferior growth was obtained. This was largely attributed to the fact that the animals scattered these fine rations which resulted in low food consumption. It can be seen in table 2 that seven of the eight animals fed the fine dextrin ration, which contained the 18% casein, died within 6 weeks. In another series when 1:20 liver extract was added to the extent of 4% and the casein increased to 24%, all four cotton rats survived the 14-week experimental period but the growth was still poor. However, all nine animals died when the soluble starch ration, which contained 4% 1:20 liver extract and 24% casein, was fed.

In contrast to these results coarse dextrin diets afforded excellent growth, in fact better growth than with any of the other carbohydrates (table 2). Animals which received the maltose, sucrose, glucose, fructose or dextri-maltose rations grew at approximately the same rate, 8.6, 9.0, 8.4, 8.0, and 7.8 gm. per week, respectively, for the first 6 weeks on experiment and 5.6 ,6.5, 6.0, 5.7 and 6.0 gm. per week for the entire 14-week period.

The incidence and extent of carious lesions were high when the soluble carbohydrates were ingested (table 3). These results are in direct contrast to the low incidence and extent observed when animals received coarse or fine dextrin rations. No appreciable difference was demonstrated between the coarse and fine dextrin rations. The replacement of 50% of the sucrose by fine dextrin did not reduce the severity of the tooth damage as compared to that observed when the sucrose basal

TABLE 2

Growth of cotton rats fed various diets.

(All results expressed as growth of males for reasons given in the text.)

RATION	NO. OF ANIMALS	RATE OF GROWTH PER WEEK	
		6 weeks	14 weeks
		gm.	gm.
801[1]	13	6.7	4.9
801 + 4% 1: 20 liver extract (L.E.)	7	8.1	6.1
801 + alcohol — ether extract ≅ to 9% 1: 20 L.E.	8	6.2	4.1
801 + alcohol extract ≅ to 6% 1: 20 L.E.	3	7.7	6.5
801 + 4% whole liver substance	2	10.3	..
801 + additional vitamins ADEK	9	4.8	3.2
Sucrose replaced by coarse dextrin	9	6.4	3.1
Sucrose replaced by fine dextrin	8[2]	2.3	..
802	32	6.9	4.5
802 + 4% 1: 20 L.E.	31	9.0	6.5
802 + 4% Sol. L.E.	7	8.2	5.8
802 + alcohol extract ≅ to 6% 1: 20 L.E.	8	7.8	4.7
802 + acetone extract ≅ to 6% 1: 20 L.E.	3	6.7	..
803 (soluble starch) + 4% 1: 20 L.E.	9[3]	..	..
804 (glucose) + 4% 1: 20 L.E.	7	8.4	6.0
805 (dextri-maltose) + 4% 1: 20 L.E.	5	7.8	6.0
806 (½ of the sucrose replaced by lactose) + 4% 1: 20 L.E.	4	6.1	4.2
807 (½ of the sucrose replaced by fine dextrin) + 4% 1: 20 L.E.	4	7.8	6.1
808 (coarse dextrin) + 4% 1: 20 L.E.	5	10.1	8.2
809 (fine dextrin) + 4% 1: 20 L.E.	4	7.0	4.4
810 (maltose) + 4% 1: 20 L.E.	5	8.6	5.6
811 (fructose) + 4% 1: 20 L.E.	4	8.0	5.7
812 (¾ of the sucrose replaced by fine dextrin) + 4% 1: 20 L.E.	4	7.8	4.0
Steenbock stock ration	6	8.3	5.0
Coarse stock ration	3	6.2	
Fine stock ration	3	5.3	
Dog Food stock ration	13	9.0	4.3

[1] Percentage composition of diet 801: sucrose 73, casein 18, salts IV 4, corn oil 5; adequate quantities of B vitamins were provided as supplements (McIntire, Schweigert and Elvehjem, '44).

[2] Seven out of 8 animals died in 6 weeks.

[3] All animals died within 3 weeks after they were placed on experiment.

ration was fed. The replacement of 75% of the sucrose by fine dextrin afforded partial protection. Apparently one-sixth of the diet as sucrose is sufficient to cause a relatively high incidence of caries.

It can readily be seen that the number of lesions observed when the control diet (802 + 4% 1:20 liver extract) was fed varied somewhat between groups; the average incidence observed in this series of experiments ranged from 23.5 to 33.0 caries per rat. These values are

TABLE 8

The effect of different carbohydrates on the incidence and extent of carious lesions.

RATION	NUMBER OF ANIMALS	AVERAGE INCIDENCE OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS
802 + 4% 1:20 liver extract (L.E.)	2	32.5	112 +
808 (coarse dextrin) + 4% 1:20 L.E.	2	5.5	10 +
809 (fine dextrin) + 4% 1:20 L.E.	4	10.3	16 +
802 + 4% 1:20 L.E.	3	25.3	74 +
807 (½ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	27.7	96 +
802 + 4% 1:20 L.E.	3	33.0	113 +
812 (¾ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	23.5	50 +
802 + 4% 1:20 L.E.	3	32.3	94 +
805 (dextri-maltose) + 4% 1:20 L.E.	3	32.7	100 +
805a (dextri-maltose + 2% NaCl) + 4% 1:20 L.E.	2	36.0	94 +
802 + 4% 1:20 L.E.	2	23.5	77 +
804 (glucose) + 4% 1:20 L.E.	7	27.6	95 +
806 (½ of sucrose replaced by lactose) + 4% 1:20 L.E.	4	22.5	64 +
802 + 4% 1:20 L.E.	3	26.3	77 +
810 (maltose) + 4% 1:20 L.E.	5	27.6	81 +
811 (fructose) + 4% 1:20 L.E.	4	29.8	92 +

of the same magnitude as observed in the earlier work. The average extent of the lesions varied from 74 + to 113 +. These differences are largely due to the variation in susceptibility to tooth decay of offspring from different parent stock. Therefore, it is important that each litter be equally represented in each group of any experiment. Records have been kept of the litters represented in each experimental series. An investigation of the susceptibility of different strains of cotton rats to caries formation is now in progress and the results from this investigation will be reported later.

DISCUSSION

The soluble carbohydrates appear to favor conditions for the formation of carious lesions while dextrin, a carbohydrate which is not readily fermented by lactic acid bacteria or other acid-producing organisms, does not favor such conditions. Unfortunately animals which received the soluble starch did not survive the experimental period, but this carbohydrate would not be expected to favor conditions for extensive tooth decay. The replacement of one-half of the sucrose by dextrin did not result in a decrease in the caries incidence. A similar result was obtained with dextri-maltose, which contains approximately 50% dextrin. Since the carbohydrate was the only dietary constituent varied in these experiments, its importance in the etiology of dental caries in the cotton rat must be emphasized.

Boyd ('42) concluded that carious lesions in children were arrested and prevented by the ingestion of diets whose quality was significantly superior to the diet of the average child. The ingestion of considerable quantities of sugar did not result in an extension of the caries or development of new carious lesions in a few cases where the effect of high quantities of sugar was specifically studied. In a later paper Boyd ('43) reported similar results from a study conducted over a 17-year period on the incidence and progression of carious lesions in children with diabetes mellitus. These experiments show that dental caries in humans can be controlled by the diet. Jay ('40) has suggested that the carbohydrate foods in the diet are largely responsible for decayed teeth.

The mechanism by which soluble carbohydrates favor conditions for a high caries incidence in the cotton rat may be much more complex than merely affecting conditions in the oral cavity. More extensive studies and many approaches to the problem will be necessary before the role of the dietary carbohydrates can be accurately evaluated.

SUMMARY

1. Data on the growth rate of cotton rats maintained on various carbohydrate diets and purified rations are presented. Additional growth responses could be demonstrated when either solubilized liver, 1:20 liver extract or whole liver substance was added to the sucrose rations.

2. Dextri-maltose, glucose, fructose, maltose and sucrose diets afforded approximately the same rate of growth. Fine dextrin, stock or soluble starch rations when fed to cotton rats produced inferior growth as compared to the sucrose control ration.

3. A very high incidence and extent of carious lesions were noted when glucose, dextri-maltose, fructose, maltose or lacto-sucrose diets were fed. However, fine dextrin and coarse dextrin diets did not favor conditions for severe tooth decay and a very low incidence and extent of the lesions were observed. There was no appreciable difference in the results obtained with coarse or fine dextrin.

4. The replacement of one-half of the sucrose with fine dextrin did not reduce the severity of tooth decay as compared to animals which received the sucrose diets. The replacement of three-quarters of the sucrose with fine dextrin reduced the incidence and extent to a small degree.

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THE RELATION BETWEEN ABSORBED NITROGEN, NITROGEN BALANCE AND BIOLOGICAL VALUE OF PROTEINS IN ADULT DOGS [1]

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TWO FIGURES

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The biological value of a protein is generally defined as the fraction of absorbed food nitrogen retained in the body of an animal, a concept proposed by Thomas and developed primarily by Mitchell ('24, '44). An objection to the use of this value has been the calculation of it which involves the assumption that excretion of nitrogen from "endogenous" sources is constant (Mitchell, '44). This concept of biological value, however, is fundamentally a function of the relationship between nitrogen balance and nitrogen intake, a relationship, which, without assumptions, can be used to evaluate dietary proteins. One of the most direct approaches to the establishment of a correlation between nitrogen balance and nitrogen intake was made by Melnick and Cowgill ('37) in their determination of protein minima for nitrogen equilibrium in dogs. They found a linear relationship in the region of nitrogen equilibrium between nitrogen balance and per cent of protein calories in the diet. The following work was done on normal adult dogs to establish more clearly over a wider range of values the relation between absorbed food nitrogen, nitrogen balance, and biological value of proteins.

METHODS

Table 1 records the protein free diet used in these studies; it is a diet similar to the one used by Melnick and Cowgill ('37). Except when otherwise noted the dogs received 80 cal./kg. of body weight daily. Protein was included in the diet by replacing equivalent amounts of calories from glucose and/or dextrin. When a natural foodstuff containing carbohydrate and fat as well as protein was added, the diet was

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adjusted so that the caloric intake was still constant and the relative proportions of constituents not altered significantly. The flounder and chicken entrails and the flounder heads used in these studies were collected fresh, ground, cooked for 1 hour in a pressure cooker at 10 pounds steam pressure, cooled and then frozen. The hearts, gizzards and livers were removed from the chicken entrails, the remainder being washed free from ingesta before grinding and cooking.

TABLE 1

Composition of protein free diet.

PROTEIN FREE DIET			VITAMIN SUPPLEMENTS	
	Per kilogram body weight			
	cal.	*gm.*		*mg./kg./day*
Sucrose	14.0	3.50	Thiamine	0.025
Dextrin	12.6	3.15	Riboflavin	.025
Glucose	21.4	5.35	Nicotinic acid	.250
Lard	32.0	3.55	Calcium pantothenate	.200
Salt [1]	...	0.30	Pyridoxine	.015
Agar	...	0.40	Choline	15.0
	80.0	16.25 [2]	2-Methyl-Naphthoquinone	.00001
			Calcium α-tocopherol monosuccinate	1.0
			Navitol (Squibbs)	I.U.: 4700 of Vit. A I.U.: 850 of Vit. D

[1] Wesson's modified Osborne-Mendel salt mixture.

[2] Mix 1.4 gm. of water with every gram of dog food.

The rations were fed for 8 days, the feces (with markers) and urine being collected the last 4 days. The feces and the urine samples were pooled respectively for each dog and analyzed for nitrogen by the Pregl micro Kjeldahl method.

Six adult dogs, parasite free, in good health and weighing from 6 to 10 kg. were used in these experiments.

RESULTS

The relationship between nitrogen balance and absorbed nitrogen when casein was the source of dietary nitrogen for the six dogs is illustrated in figure 1-A. Both nitrogen balances and absorbed nitrogen values are expressed as grams per day per square meter of body surface. The relationship is linear in the region of negative balance, the linearity extending over into the positive side but becoming obviously curvilinear well on the positive side of nitrogen balance.

The empirical equation for the linear portion of this relationship is,

$$NB = k\ (AN) - NE_o \qquad (1)$$

where NB is nitrogen balance, AN absorbed nitrogen, NE_o the excretion of nitrogen on the protein free diet, and k the slope of the line. Thus the slope of the line is the rate of change of nitrogen balance with respect to absorbed nitrogen, and is one measure of the biological value of the protein. This slope is constant over the linear portion becoming a variable which decreases with increasing nitrogen intake over the curvilinear portion of the curve.

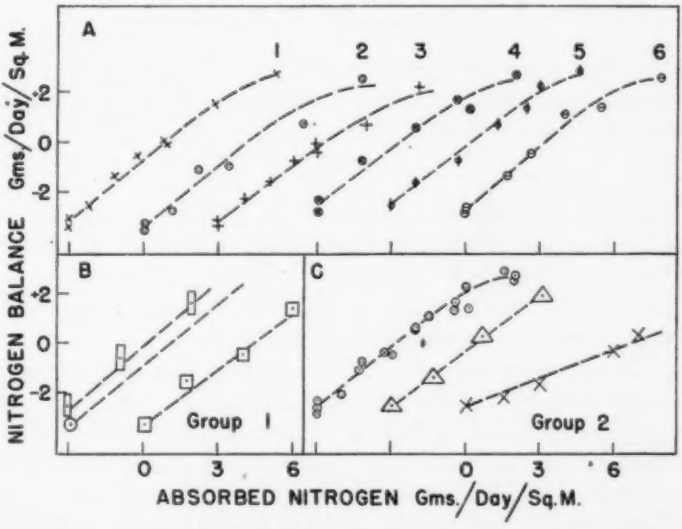

Fig. 1 A plot of absorbed nitrogen vs. nitrogen balance. All curves originate at 0 absorbed nitrogen. Figure 1-A describes data obtained on dogs 1–6 fed casein as the source of nitrogen. Figure 1–B and C describes average data obtained on dogs 1, 2, and 3, labeled group I and 4, 5, and 6, labeled group II. See text for further description.

If certain assumptions are made, the slope or k in equation (1) is the fraction of absorbed food nitrogen retained in the body of an animal, the biological value as generally defined. This definition of biological value (BV) can be illustrated by equation 2.

$$BV = \frac{AN - (UN - UN')}{AN} \qquad (2)$$

where AN is absorbed nitrogen, UN the excretion of total urine nitrogen and UN′, the excretion of urine nitrogen of "endogenous" sources.

Absorbed nitrogen is calculated according to equation 3

$$AN = NI - (FN - FN') \tag{3}$$

where NI represents nitrogen intake, FN total feces nitrogen, and FN′ feces nitrogen of "endogenous" origin.

The equivalent of AN in equation 3 can be substituted into the numerator of 2, so that,

$$(BV)\ (AN) = NI - FN - UN + FN' + UN' \tag{4}$$

Since by definition,

$$NB = NI - FN - UN \tag{5}$$

then,

$$(BV)\ (AN) = NB + UN' + FN' \tag{6}$$

Assuming that the total excretion of nitrogen on a protein free diet (NE_o) is equal to the sum of nitrogen of "endogenous" origin in urine and feces ($UN' + FN'$), then,

$$NB = (BV)\ (AN) - NE_o \tag{7}$$

Equation 7 is identical to equation 1 except that k in 1 now has the definition of biological value generally used. Thus, if the assumption is made that "endogenous" nitrogen excretion is constant, then the slope of the line relating nitrogen balance to absorbed nitrogen becomes the fraction of food nitrogen retained in the body.

Values for nitrogen excretion on the protein free diet together with absorbed nitrogen at equilibrium and biological values for each of the six dogs are recorded in table 2. The biological values recorded in the last column in the table may be considered constant, since the deviations from the mean are not significant. These values are the slopes of the lines in figure 1-A. If it is assumed that "endogenous" nitrogen is constant, they represent the fraction of absorbed nitrogen retained by each animal; otherwise they are simply the rate of change of nitrogen balance with respect to absorbed nitrogen. Under absorbed nitrogen at equilibrium in the third column, are values which represent the protein minima for nitrogen equilibrium. These minima are approximately 4 gm. for the first three dogs and 3.1 gm. for the second three. The difference in minima between the two groups of dogs is correlated with the greater excretion of nitrogen on the protein free diet by dogs 1, 2, and 3, than by 4, 5, and 6, which can be interpreted to mean that the first three dogs were excreting more nitrogen from body stores requiring more absorbed protein nitrogen to maintain equilibrium. These observations agree with those of Melnick and Cowgill ('37) who noted that the lines relating nitrogen balance to per cent protein caloric

intake tended to be parallel, while protein minima varied. More recent experiments have shown that increasing the caloric intake by adding carbohydrate to the diet of dogs 1, 2, and 3 decreases the excretion of nitrogen on a protein free diet bringing the data into line with the other three dogs.

The first two curves in figure 1-B illustrate this effect of increasing the caloric intake of dogs 1, 2, and 3, labeled group I. The second dotted line originating at the circle represents the average curve for the data on the first three dogs fed casein in a diet of 80 calories per kilogram of body weight (figure 1-A). The first line through the rectangles is an average curve for the same three dogs when the caloric intake was increased to 100 calories per kilogram of body weight. The slopes, and

TABLE 2

Nitrogen excretion on a protein free diet (NE_o), absorbed nitrogen at nitrogen equilibrium and the biological value (BV) taken from each curve in figure 1-A. Casein was the source of dietary nitrogen.

DOG NO.	PROTEIN FREE NITROGEN EXCRETION NE_o	ABSORBED NITROGEN AT EQUILIBRIUM	BIOLOGICAL VALUE BV
	gm./day/sq.M.	*gm./day/sq.M.*	
1	3.2	3.8	0.84
2	3.4	4.0	0.85
3	3.3	4.2	0.78
4	2.5	3.1	0.81
5	2.5	3.1	0.81
6	2.7	3.2	0.84
		Average	0.82

therefore the biological values of casein at the two caloric intakes, are identical. The only change is a shift in the excretion of nitrogen, being lower on the higher caloric intake, thus demonstrating the protein sparing action of carbohydrate. The fact that the excretion of nitrogen can shift without altering the biological value supports the concepts developed by Mitchell ('24, '44).

The first curve in figure 1-C averages the same data for casein obtained on dogs 4, 5, and 6, labeled group II, as plotted in figure 1-A. The slope of this line is 0.82 the same as the slopes of the lines for casein in group I. The line through the triangles describes average data obtained using chicken entrails as the source of protein nitrogen in dogs 4, 5, and 6. The slope is 0.77, which is slightly less than that for casein. The line through the crosses represents data for a commercially prepared protein derived from soybean, with a slope of 0.39, much lower

than the others. The squares in the figure 1-B summarize data obtained on dogs 1, 2, and 3, using flounder entrails as the source of protein in the diet, the slope of the line being the same as that for chicken entrails. Table 3 summarizes some of these and other averaged data on dogs in groups I and II.

TABLE 3

Digestibilities, absorbed nitrogen at nitrogen equilibria, and biological values (BV) of casein, protein A and proteins in chicken entrails, flounder entrails, and flounder heads. Data are averages from determinations on two groups of three dogs each, I and II.[1]

DOG GROUP	PROTEIN SOURCE	TRUE DIGESTIBILITY	ABSORBED NITROGEN AT EQUILIBRIUM	BV
		%	*gm./day/sq.M.*	
I	Casein	96	4.0	0.82
I[1]	Casein	95	3.2	0.81
II	Casein	95	3.1	0.82
II	Chicken entrails	94	3.3	0.77
I	Flounder entrails	96	4.3	0.77
I	Flounder heads	89	6.4	0.52
II	Protein A[2]	82	6.6	0.39

[1] All dogs received 80 calories per kilogram of body weight except those in the experiments in this group when 100 calories per kilogram of body weight were fed.
[2] Derived from soybean.

Equation 1 is useful, therefore, in determining directly the biological value and the protein minima for nitrogen equilibrium. But the relationship between urine nitrogen excreted and absorbed nitrogen is also of fundamental interest. This relationship can be derived from equation 1 or 7 in the following way. The symbols are the same as those used previously.

$$NB = AN + FN - FN' - UN - FN \tag{8}$$

Substitute 8 in 7, expanding NE_o in 7 to $UN_o + FN_o$, so that,

$$AN - FN' - UN = (BV)\ (AN) - UN_o - FN_o \tag{9}$$

Assume $FN_o = FN'$ then,

$$UN = (1 - BV)\ AN + UN_o \tag{10}$$

Where equation 1 or 7 described a linear relationship between NB and AN, equation 10 also describes a linear relationship between UN and AN as illustrated in figure 2. The symbols used in figure 2 are the same as those in figure 1-B, C. The circles in the lower left hand part of figure 2 describe the same data for casein as illustrated in figure 1-C for dogs in group II. Similarly the triangles and the crosses describe respectively data for chicken entrails and the derived soybean proteins.

The squares illustrate the data obtained on dogs in group I when they were fed fish entrails as the source of protein nitrogen. The slopes of the lines in this figure are equal to (1 — BV). When BV is equal to unity then urine nitrogen excretion would be independent of absorbed nitrogen, the excretion remaining the same as on a protein free diet.

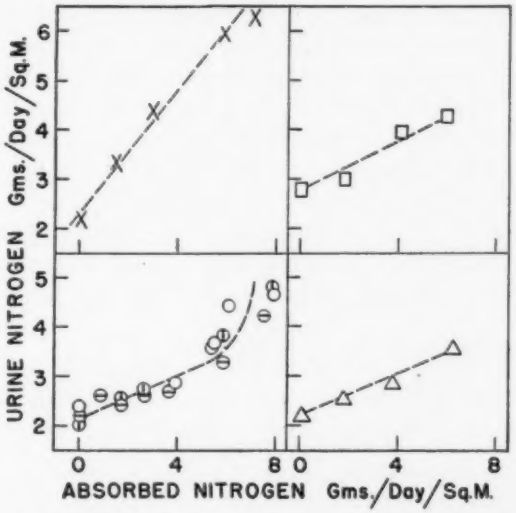

Fig. 2 A plot of absorbed nitrogen vs. urine nitrogen using data from the same experiments illustrated in figure 1–B and C.

Under these conditions UN_o in equation 10 could be determined without feeding a protein free diet. If biological value is the fraction of nitrogen retained in the body of an animal, then BV can never be greater than unity, since the retention of more than 100% of the absorbed nitrogen is an impossibility. On the other hand, if "endogenous" nitrogen metabolism is not constant, BV will not represent the fraction of absorbed nitrogen retained and could be greater than unity. Under these conditions urine nitrogen could decrease as absorbed nitrogen increases, an example of food nitrogen sparing body nitrogen.

SUMMARY

1. The relationship between nitrogen balance and absorbed nitrogen in normal adult dogs is linear in the region of negative nitrogen balance, the linearity often extending over onto the positive side, but becoming obviously curvilinear well on the positive side of nitrogen balance.

2. The equation describing the linear portion is,

$$NB = k\ (AN) - NE_o$$

where NB is nitrogen balance, AN absorbed nitrogen, NE_o the excretion of nitrogen on a protein free diet and k the slope of the line. If the excretion of nitrogen of endogenous origin is constant, k is the fraction of nitrogen retained in the body of an animal. This fraction is the customary definition for the biological value of a protein.

3. When the caloric intake was reduced below optimum, excretion of nitrogen on a protein free diet (NE_o) and protein minima for nitrogen equilibrium increased, but the biological value (k) was not altered. Thus reduced caloric intake increased the utilization of body nitrogen but did not alter the utilization of dietary nitrogen.

4. The value for k was determined for a variety of protein sources in the adult dog as follows: casein = 0.82, chicken entrails = 0.77, flounder entrails = 0.77, flounder heads = 0.52, and a protein derived from soybean = 0.39.

5. The relationship between urine nitrogen (UN) and absorbed nitrogen (AN) was shown to be,

$$UN = (1 - BV)\ AN + UN_o$$

where BV is biological value, and UN_o the excretion of urine nitrogen on a protein free diet.

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NUTRITIONAL VALUE OF YEAST PROTEIN TO THE RAT AND THE CHICK

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This laboratory (Stubbs, Noble, and Lewis, '44; Lewis, Stubbs, and Noble, '44) has been interested for some time in the utilization of cannery wastes for the production of yeast for use in feeds.

One phase of this project has been the nutritional evaluation of the protein of the yeast produced. Feeding experiments with rats recently reported from this laboratory (Klose and Fevold, '44) have shown that yeast, when fed as the sole source of protein at levels as high as 13% crude protein, supplies an inadequate amount of methionine for optimum growth in the young rat. This work has been confirmed and extended in a series of rat and chick growth tests, the results of which are given here.

The literature concerned with the amino acid content and nutritional value of the proteins of yeasts has been adequately reviewed by Carter and Phillips ('44). However, some of the recent work that bears most directly on our studies will be mentioned here. Temperton and Dudley ('41) fed groups of thirty laying pullets a practical cereal ration in which the protein concentrate was supplied by fish meal (10%), torula yeast (12%), or brewer's yeast (12%). No differences between the three groups were observed as regards egg production, general health, or body weight. Feeding experiments with poultry, summarized by Axelsson ('41), indicated that at least half of the usual quantity of animal protein can be replaced by yeast protein.

Growth tests with rats were carried out by Hock ('42) to determine to what extent animal protein can be replaced by yeast protein. Determinations of the average gain in weight per gram of protein consumed were made for rats on diets containing 9.3% crude protein, of which 1.5% was derived from cereals and 7.8% from various combinations of fish meal and yeast. Essentially the same results were obtained for brewers' yeast and for torula yeast grown on wood-sugar. The growth

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rate was not affected appreciably by substitution of either brewers' yeast or torula yeast protein for half of the fish-meal protein; 75% replacement reduced the gain 15 to 25%, while complete replacement of fish meal by yeast resulted in a 50% decrease in weight gain per unit of protein consumed.

A recent report by Hock and Fink ('43) describes rat-growth tests in which yeast protein was beneficially supplemented with cystine. The percentage of total crude protein in the diets was in most cases 10%, of which 1.6% was furnished by cereals and the remainder (8.4%) by either brewers' yeast or wood-sugar yeast. Addition of 0.2% of cystine to the diets containing yeast resulted in an approximate doubling of the growth rate. The cystine-supplemented yeast diet had a growth-promoting effect nearly equal to that of a diet in which 90% of the yeast protein was replaced by milk protein. During the experimental period of 90 days, all of the yeast-fed groups experienced liver damage and heavy mortality. However, the mortality was considerably lower in the groups that received cystine.

The amounts of the essential amino acids in whole yeast, reported by Block and Bolling ('43)[2] as percentages of the crude protein ($N \times 6.25$), were as follows: arginine 4.3, histidine 2.8, lysine 6.4, tryptophane 1.4, phenylalanine 4.1, threonine 5.0, leucine 13.2, isoleucine 3.4, valine 4.4, cystine 1.3, and methionine 2.0. Values for the percentage of cystine in the crude protein ($N \times 6.25$), of dried whole yeast ranging from 0.6 to 2.2 have been reported (Prunty, '33; Csonka, '35; Fink and Just, '42). Reporting on the basis of crude protein ($N \times 6.25$) neglects the presence of nonprotein nitrogen (purines, pyrimidines, etc.), which has been calculated from Van Slyke nitrogen fractionations, and other means, to represent approximately 15 to 20% of the total nitrogen present in yeast. Most nutritional data have been expressed on the basis of crude protein content, since the available information is insufficient to characterize completely a large majority of protein concentrates in terms of their component proteins and nonprotein nitrogen constituents. It appears advisable in the present work to adhere to this conventional basis of comparing the nutritional value of protein concentrates, at the same time keeping in mind the discrepancies involved and the corrections that may be applied when more data become available.

EXPERIMENTAL WORK

All sources of protein were fed on the basis of their crude protein content (total Kjeldahl nitrogen $\times$ 6.25). The yeast sample designated

[2] Also see Melnick, '43.

as "brewers' yeast" was a commercial type grown on a hop-free grain medium. It had a crude protein content of 44%. Torula yeast, sample A,[3] consisted of Torulopsis utilis grown on a prune-juice medium. The yeast cells were separated by pressing, dried in ribbon form in a tunnel dehydrator, and then ground in a hammer mill. Torula yeast sample B represented a similar product prepared from blackstrap molasses. Samples A and B contained 57.5 and 53.2% crude protein, respectively. Sample B was found to contain 0.1% cystine.[4] Of the reference proteins used, casein had 84%, sardine meal 70%, and soybean meal 44% crude protein. A 50% methanol extract of brewers' yeast was prepared by leaching the yeast with three successive volumes (2.5 ml. of solvent per gram of yeast) of the extractant and concentrating in vacuo to a thick syrup. This protein-free concentrate was added to those diets that contained little or no yeast in order to minimize the possibility of an uncompensated growth effect from some nonprotein factor in the yeast.

Growth tests with rats

The general procedure used in all of the rat-growth experiments to be described was as follows: Albino rats of the Sprague-Dawley strain were maintained on a stock diet of Purina Dog Chow pellets, supplemented with cod liver oil and lettuce, until the experimental diets were fed. All experimental groups contained equal numbers of males and females. Feed and water were furnished ad libitum, and a record was kept of the daily feed consumption for each cage. The rats were weighed individually at frequent intervals and examined at the end of the experimental periods for gross manifestations of toxic effects.

Preliminary feeding experiments with young rats had shown that a dietary level of 28% torula yeast A gave only one-third of the growth rate produced by a 16% level of commercial casein. Therefore experimental diets were designed to test the adequacy of yeast protein when fed as the sole source of protein and when fed in combination with casein. The ten groups of experiment A, each consisting of sixteen, 38-day-old rats, were placed on the diets described in table 1. Groups IV a and VII a were limited to a test period of 10 days; all other groups of experiment A were maintained on their respective diets for a 5-week period. The average initial weight of the rats of all groups was about

[3] The samples of torula yeast were made available through the generous cooperation of Dr. E. C. Saudek and Mr. W. M. Noble of Consumers Yeast Company and Drs. I. C. Feustel, H. Humfeld and J. C. Lewis of this laboratory.

[4] We are indebted to D. K. Mecham of this laboratory for the analysis. A modification of Vassel's method was used (Mecham, '43).

73 gm. No deaths occurred during the experimental periods. A denudation, appearing first around the ears and later extending over the shoulders and back, was noted in the rats of groups IV and VII after 3 weeks on the experimental diets. Otherwise all rats were healthy and showed no macroscopic evidence of toxic effects upon being sacrificed

TABLE 1

Comparative growth promoting effect of protein from yeast and from casein.

	EXPERIMENT A									
	Diet no.									
	I	II	III	IV	IVa	V	VI	VII	VIIa	VIII
Basal mixture,[1] %	65	65	65	65	65	65	65	65	65	65
Starch-sucrose (50-50), %	19	23	27	5	5	12	15.5	10	10	14.5
Yeast extract (protein-free), %	≅ 5	≅ 5	≅ 5	..	..	..	..	..	..	..
Commercial casein, %	16	12	8	..	..	8	12	..	..	8
Brewers' yeast, %	..	..	..	30	30	15	7.5	..	..	..
Torula yeast B, %	..	..	..	..	..	..	..	25	25	12.5
dl methionine, %	..	..	..	..	0.5	..	..	..	0.5	..
Crude protein from casein, %	13.4	10.0	6.7	..	..	6.7	10.0	..	..	6.7
Brewers' yeast, %	..	..	..	13.2	13.2	6.6	3.3	..	..	..
Torula yeast B, %	..	..	..	..	..	..	..	13.3	13.3	6.7
Average gain in weight, gm./rat/day[2]	2.5	1.6	0.4	0.6	3.3	1.9	2.3	0.6	3.2	2.0
Feed consumption, gm./rat/day	10.9	10.5	8.1	9.1	9.3	10.9	10.4	8.2	9.8	11.0
Gm. gain/gm. crude proteins consumed	1.71	1.54	0.68	0.47	2.70	1.30	1.69	0.58	2.5	1.33

[1] The composition of the basal mixture used in the diets was as follows: As per cent in whole diet, sucrose 25, corn starch 25, cottonseed (Wesson) oil 10, salt mixture (McCollum's no. 185) 4, U.S.P. cod-liver oil 1, choline chloride 0.05; and as mg. per 100 gm. in whole diet, thiamine chloride 0.2, riboflavin 0.5, pyridoxine 0.2, calcium pantothenate 2.5 and nicotinic acid 1.0.

[2] Comparable groups of rats on stock colony diet gained 3.5 gm./rat/day.

and examined at the conclusion of the test. The resultant growth data given in table 1 demonstrate that, at a total crude protein level of 13.4%, neither of the two yeasts tested could support growth comparable to that obtained with an equivalent amount of crude casein protein. One-fourth of the casein, however, could be replaced by yeast without appreciable effect on growth rate. The good growth obtained when yeast was supplemented with methionine (groups IV a and VII a) demonstrates the nature of the deficiency in yeast protein.

A second rat growth test (table 2, experiment B) was set up in order to determine to what extent cystine could correct the methionine deficiency in yeast protein, and to make further comparisons between casein and the yeast protein. Each group contained six males and six females, with an average initial age of 40 days and average initial weight of 83 gm. Experimental diets were fed over a period of 37 days.

TABLE 2

Growth responses obtained with methionine and cystine supplements to a yeast diet, compared with responses with reference casein diets.

	RAT EXPERIMENT B									
	Diet no.									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Basal mixture,[1] %	60	60	60	60	60	60	60	60	60	60
Starch-sucrose (50-50), %	15	15	15	15	15	..	27	24	24	20
Yeast extract (protein free), %	..	..	..	..	..	..	≅ 5	≅ 5	≅ 5	≅ 5
Commercial casein, %	..	..	..	..	..	..	13	16	16	20
Torula yeast B, %	25	25	25	25	25	40	..	..	..	..
l-cystine, %	..	0.5	..	..	0.5	..	..	..	0.2	..
dl-methionine, %	..	..	0.2	0.5	0.2	..	..	..	..	..
% crude protein, %	13.3	13.6	13.4	13.6	13.7	21.3	10.9	13.4	13.5	16.7
Average gain, gm./rat/day	0.8	1.0	2.0	2.4	2.7	1.7	2.0	2.7	3.1	3.1
Feed consumption, gm./rat/day	8.6	7.8	9.2	9.5	10.2	9.3	12.0	11.7	11.6	11.6
Gm. gain/gm. crude proteins consumed	0.73	0.91	1.65	1.82	1.90	0.84	1.54	1.70	1.96	1.58

[1] The composition of the basal mixture used in the diets of experiment B was as follows: As per cent in whole diet, sucrose 22, corn starch 22, cottonseed (Wesson) oil 10, salt mixture (McCollum's no. 185) 4, U.S.P. cod liver oil 2, vegetable oil concentrate containing 40% tocopherols 0.05, choline chloride 0.05; and as mg. per 100 gm. in whole diet, thiamine chloride 0.2, riboflavin 0.5, pyridoxine 0.2, calcium pantothenate 2.5, and nicotinic acid 1.0.

Yeast protein at levels of 13.3 or even 21.3% crude protein again was found inadequate for optimum growth. Supplementation with 0.5% methionine at the 13.3% crude protein level resulted in essentially the same rate of growth as was obtained with a 13.4% crude protein level of casein. Cystine had a definite supplementing effect on yeast protein, which was most striking in the group fed diet V.

A comparison of the growth rates in group VIII, IX and X demonstrates the long recognized fact that casein, when used in diets at levels below 18% crude protein, supplies a suboptimal amount of cystine.

Very little of the denudation noted in experiment A occurred in the groups of experiment B. As in earlier tests, no deaths occurred during the experimental period, in contrast to the results of Hock and Fink ('43), who reported considerable mortality during the first 5 weeks of a 90-day test period. At the conclusion of experiment B, the rats were sacrificed and examined macroscopically for fatty livers or other abnormal conditions. No liver damage or other organic lesions were apparent.

A feeding experiment with rats on a 37.5% torula yeast diet over a period covering the growth of two generations was still in progress when this paper was written. No deaths had occurred in male rats after 75 days on this diet, or in females after 90 days. Sacrifice and examination of the males after 75 days showed no abnormalities in the liver or kidneys.

Chick growth tests

Possible uses of yeast include its substitution for animal protein in poultry rations. Since the amino acid requirements of the chick are known to differ in some respects from those of the rat, it was considered desirable to investigate the effect of adding yeast to chick diets in amounts that would furnish significant contributions of proteins.

In the chick growth tests to be described, 1-day-old single-comb White Leghorn chicks were housed in thermostatically controlled, electrically heated, all-metal battery brooders and fed an adequate practical chick ration until the feeding experiments were started. Groups of chicks, selected for the same average weight and weight distribution, were fed the experimental diets ad libitum during a 12-hour day controlled arbitrarily by lighting. All diets were adjusted to contain approximately 1.6% calcium and 1.0% phosphorus. Records were kept of the individual chick weights and of the feed consumption by groups.

Chick test I. Preliminary growth studies with chicks demonstrated that 80% of the protein of the fish meal in a practical chick diet could be replaced by yeast protein without seriously affecting the growth rate or efficiency of food utilization. It was thought desirable to compare the growth-promoting value of protein from yeasts with those of fish-meal and soybean-meal protein, especially when each occurred in a cereal diet as the sole protein concentrate. Four hundred 1-day-old chicks of both sexes were maintained on an adequate practical chick ration (diet 1 in table 3) for 10 days. Then nine groups of 28 chicks each, selected from those ranging from 70 to 90 gm. in weight, were fed the diets described in table 3 for 20 days.

The resultant growth data in table 3 indicate that when used as the sole source of noncereal protein, yeast is markedly inferior to sardine meal and significantly inferior to soybean meal. This is in contrast to the satisfactory results obtained by replacing 80%, rather than 100%, of the protein of sardine meal by yeast protein. Although the differences are not highly significant, it may be pointed out that the combination of soybean meal and sardine meal appeared superior to

TABLE 3

A comparison of the growth produced by yeast, sardine meal, and soybean meal in chick diets.

INGREDIENT	CHICK TEST I								
	Diet no.								
	1	2	3	4	5	6	7	8	9
Basal mixture,[1] %	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5	71.5
Ground corn, %	11	10	10	..	6	4	..	5	2
Corn starch, %	..	5	10	..	..	..	..	..	..
Steamed bone meal, %	1.3	2.3	3.2	2.6	1.7	1.4	3.1	2.2	2.3
Calcium carbonate, %	1.0	0.8	0.7	1.7	2.5	2.7	1.2	1.1	2.0
Yeast extract, %	≅ 10	≅ 10	≅ 10	..	..	..	≅ 10	≅ 10	..
Sardine meal, %	15	10	5	..	..	..	..	7.5	..
Brewers' yeast, %	..	..	..	24	..	..	..	..	..
Torula yeast A, %	..	..	..	..	18	..	..	..	..
Torula yeast B, %	..	..	..	..	..	20	..	..	10
Soybean meal, %	..	..	..	..	..	..	24	12	12
Crude protein, %	19.8	16.2	13.1	19.1	19.8	19.5	19.0	19.6	19.5
Gain in weight, gm./chick/day									
Average	11.8	10.3	8.2	8.3	9.1	8.9	10.9	12.0	9.9
Stand. error	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.5	0.3
Feed consumption, gm./chick/day	25	26	23	32	30	26	28	28	29
Feed utilization, gm. gain/gm. feed	0.46	0.40	0.36	0.26	0.30	0.33	0.39	0.44	0.35

[1] The basal mixture provided, as per cent of the complete diets: ground whole corn 30, ground whole wheat 10, ground oat groats 10, wheat bran 15, alfalfa meal 5, sodium chloride containing 2% manganese sulfate 0.5, and U.S.P. cod liver oil 1.

the soybean meal alone, as has been reported by Hammond and Titus ('44). However, the combination of soybean meal and torula yeast gave a lower growth rate than the soybean meal alone. This is understandable in view of the relative methionine shortages in the two protein concentrates.

Chick test II. A second set of feeding experiments was set up to study the methionine deficiency in yeast protein when fed as the sole source of protein in simplified chick diets.

One-day-old cockerels were fed an adequate practical chick ration for 10 days. At this time the chicks were selected for uniformity, wing-banded, and divided into groups of ten each. The average weight gains and feed consumption corresponding to the various supplements are given in table 4. It is apparent that torula yeast fed at a 40% level (21.3% crude protein) was not supplying an adequate amount of methionine for optimum growth. This condition could be corrected to varying degrees by supplements of 0.5% methionine, 1% cystine, or 5% dried egg white. It may be estimated from analyses reported in the literature that the dried egg white contributed approximately 0.2% methionine and 0.1% cystine to the diet.

TABLE 4

The effect of amino acid and protein supplements on the growth rate of chicks fed a 40% yeast diet.

		CHICK TEST II					
			Average gain gm./chick/day				
Diet no.	Supplement to 40% yeast diet [1]	Days on diet	Aver.	±	Stand. error	Feed consumption gm./chick/day	Feed utilization gm. gain/ gm. feed
1	None	1–16	4.4	±	0.6	27	0.16
1a	None	1–6	4.8	±	0.6	20	0.23
2	1% glycine	7–16	4.1	±	0.7	24	0.17
3	1% cystine	1–16	7.1	±	0.6	22	0.32
4	0.5% methionine	1–16	9.8	±	0.7	24	0.40
5	5.0% dried egg white	1–14	8.5	±	0.6	24	0.35

[1] This diet contained, as per cent: torula yeast B 40, glucose 22, corn starch 22, gum arabic 8, calcium carbonate 3, tricalcium phosphate 1, sodium chloride plus trace elements 1, potassium chloride 0.5, cottonseed (Wesson) oil 1, U.S.P. cod liver oil 1, vegetable oil concentrate containing 40% tocopherols 0.05, and 2 methyl 1, 4 naphthoquinone 0.0002.

The general health and appearance of the chicks in the three tests described in this report were good. Partly because of the severe culling at the start of the tests, no deaths occurred during any of the experimental periods. Chicks on the simplified diets were rather poorly feathered and grew at a much slower rate than those on practical rations. This is a common observation with simplified diets and probably reflects the physical nature of the diet and relatively obscure nutritional deficiencies.

DISCUSSION

On the basis of Block's value of 2.0% for the methionine content of the crude protein of yeast, and 0.2% for the cystine content, we may estimate that the 25% level of torula yeast was furnishing 0.27% of

methionine and 0.025% of cystine to the diet. Womack and Rose ('41) have set the methionine requirement in the rat at 0.6%, or 0.5% in the presence of an adequate amount of cystine. This provides a basis for a partial explanation of the results obtained by supplementing yeast with varying amounts of methionine and cystine. For example, in rat experiment B, the addition of 0.5% cystine to the 25% torula yeast diet (containing 0.27% methionine) gave only a very small increase in growth rate compared with the addition of 0.2% methionine. However, when we tested the relative supplementing effect of cystine and methionine at a higher basal level of methionine content, which approached the minimum methionine requirement (compare diets III, IV, and V in table 2, experiment B), we found that cystine served equally as well as methionine.

The high mortality in rats fed high levels of yeast as reported by Hock and Fink ('43) is in marked contrast with the 100% survival in our tests. Differences in the source of yeast, strain of rat, or basal diet could conceivably cause this discrepancy.

The experimental results of the chick growth tests agreed in general with the results of the rat studies. In simplified diets, levels as high as 40% torula yeast appeared to be deficient in methionine for optimum growth of the chick. A 40% yeast level should supply 0.42% methionine and 0.04% cystine to the diet. Almquist, Mecchi, Kratzer, and Grau ('42) have measured the chick's requirements and found them to be 0.55% methionine and 0.4% cystine, or the equivalent of these two amounts in methionine. The results of chick test II agreed with this formulation of the sulfur-containing amino acid requirements of the chick, since a 1% cystine supplement (incapable of satisfying the remaining 0.13% methionine requirement) gave a smaller increase in growth rate than did a 0.5% methionine supplement.

In practical cereal chick rations yeast protein effectively replaced as much as 80% of the animal protein concentrate. However, when animal protein was replaced completely by yeast protein, a definite decrease in growth rate occurred. In the field of protein concentrates, yeast may be compared most closely with soybean meal. Compared with good animal proteins, both of these proteins are seriously inadequate in respect to only one essential amino acid, methionine.

SUMMARY

1. Brewers' yeast and torula yeast grown on molasses have been fed as the principal or only source of protein in the diet of rats during the period of rapid growth. The same yeasts and also a torula yeast

grown on prune juice were fed to chicks. At the levels fed, and in comparison with equivalent amounts of relatively complete proteins, each of the three yeast preparations contained an inadequate amount of methionine for optimum rate of growth.

2. This inadequacy could be corrected by the addition of methionine or methionine-rich protein to the diet.

3. Cystine corrected the deficiency to a limited extent.

4. Yeasts at relatively high levels and over limited periods did not appear to be toxic.

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THE EFFECT OF FEEDING SUCCINYLSULFATHIAZOLE TO RATS RECEIVING PURIFIED DIETS HIGH IN CARBOHYDRATE, PROTEIN, FAT, OR PROTEIN AND FAT

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TWO FIGURES

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The incorporation of succinylsulfathiazole (SST) into otherwise adequate highly purified diets renders such diets incapable of supporting normal growth in the rat (Welch, '42). Accompanying the failure in growth are such evidences of a deficiency syndrome as alopecia, porphyrin-stained whiskers, spectacled eye, ophthalmitis, achromotrichia, leucopenia and agranulocytopenia (Daft et al., '42; Spicer et al., '42; Light et al., '42; Martin, '42 a, b; Nielsen and Elvehjem, '42; Welch and Wright, '43; Wright and Welch, '43). Normal growth and alleviation of the signs of deficiency may be brought about by the administration of "folic acid" and biotin. The deficiency state is characterized by low hepatic stores of "folic acid," biotin and pantothenic acid which return to adequate levels when "folic acid" and biotin are given (Wright and Welch, '43, '44).

Studies of the requirement of the rat for certain dietary factors when receiving SST in highly purified diets have been largely limited to the use of rations containing approximately 18% casein with the remainder of the diet composed largely of sucrose. The importance of the composition of the diet in relation to vitamin requirements has been demonstrated. In the case of thiamine, for example, it has been shown that more thiamine is required for normal growth with a diet high in carbohydrate than is required with diets high in protein or fat (Evans, Lepkovsky and Murphy, '34; Arnold and Elvehjem, '39; Wainio, '42). Larger amounts of riboflavin are required for normal growth of rats receiving diets high in fat (Mannering et al., '41).

The following experiments were designed, therefore, to study the relationship of the dietary composition to the deficiencies brought about

by feeding SST to rats. It was thought possible that variations in the diet might alter the intestinal flora or otherwise modify the action of SST in such a way that additional deficiencies heretofore not noted might develop. Another possibility was that alteration in the diet might indicate in what way the factors which are affected by the feeding of poorly absorbed sulfonamides function in the normal metabolism of the rat. Observations on the growth and incidence of signs of deficiency have been supplemented by studies of the number and distribution of the intestinal flora and quantitative studies of the fecal elimination and liver storage of factors known to be involved in a deficiency induced by SST.

PROCEDURE

Albino rats weighing between 35 and 45 gm. were divided into four groups of twelve each and caged individually over wire mesh screening. Diets high respectively in carbohydrate, protein, fat, or in both protein and fat were fed ad libitum (table 1). After 2 weeks, when it

TABLE 1

Composition of the diets employed.

VARIED INGREDIENTS OF BASAL MIXTURE	HIGH CARBOHYDRATE I	HIGH PROTEIN II	HIGH FAT III	HIGH PROTEIN AND FAT IV
	gm.	*gm.*	*gm.*	*gm.*
Vitamin-free casein	180	300	180	300
Sucrose	660	540	460	340
Hydrogenated cottonseed oil [1]	100	100	300	300

Each diet also contained the following (in gm.): corn oil 20, salts [2] 40, vitamins A, D, and E [3] 1, choline 1; and the following (in mg.): vitamin K [4] 10, thiamine hydrochloride 8, riboflavin 16, nicotinic acid 40, pyridoxine-hydrochloride 8 and calcium pantothenate 44.

[1] 'Primex'.

[2] Hubbell, Mendel and Wakeman ('37).

[3] Compounded as follows: Fish liver concentrate containing 450,000 U.S.P. units of vitamin A and 90,000 U.S.P. units of vitamin D per gram, 7 gm.; α-tocopherol, 2 gm.; corn oil, 41 gm.

[4] Used in the form of 2-methyl-1, 4-napthoquinone.

was felt that the rats had become accustomed to the change in diet, 5% SST replaced an equivalent amount of sucrose in each of the diets. Weights were recorded weekly and, as signs of deficiency developed, at more frequent intervals.

The bacterial flora of the feces was followed by dilution plate counts made twice a week on the feces of three rats chosen at random in each group. The total number of organisms (aerobic and anaerobic), spores (aerobic and anaerobic), streptococci, lactobacilli, and coliforms were

determined, using the differential technique of Strawinski, Verwey and Munder ('45).

Determinations of "folic acid," biotin and pantothenic acid were made on 24-hour samples of feces (pooled sample of three rats in each group) at weekly intervals. One-half of each sample was digested for 24 hours at 37°C. with 2% of its weight of takadiastase in pH 4.0 acetate buffer (Cheldelin et al., '42) prior to microbiological assay for "folic acid" [1] (Landy and Dicken, '42) and pantothenic acid (Skeggs and Wright, '44). Biotin determinations were made according to the procedure of Wright and Skeggs ('44) on the remainder of the 24-hour

TABLE 2

The fecal elimination of "folic acid," biotin and pantothenic acid in rats receiving various diets.

TREATMENT	"FOLIC ACID"				BIOTIN				PANTOTHENIC ACID			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
	μg./rat/day				*μg./rat/day*				*μg./rat/day*			
No sulfonamide [1]	2.1	1.9	1.6	2.0	0.41	0.32	0.28	0.25	20.1	16.9	21.1	19.2
5% SST [2]	0.31	0.54	0.38	0.64	0.043	0.054	0.041	0.053	10.6	20.5	15.8	20.7

I = high carbohydrate diet; II = high protein diet; III = high fat diet; IV = high protein and fat diet.

[1] Results given are the average of two determinations made on the 8th and 11th days of feeding the purified diets.

[2] Results given are the average of seven determinations made at weekly intervals after the inclusion of SST in the diets.

samples after they had been autoclaved for 1 hour with 6 N H_2SO_4. In all the microbiological assays of feces p-aminobenzoic acid was included in the assay medium (10 mg. per 100 ml.) as a sulfonamide inhibitor.

Representative rats from each group were sacrificed at intervals throughout the feeding period and at the end of the experiment to obtain data on the liver storage of "folic acid," biotin and pantothenic acid. One-half of each liver was prepared for microbiological assay for "folic acid" and pantothenic acid by digestion in water with takadiastase (Wright, Skeggs and Welch, '45). The remaining portion of each liver was autoclaved with 6 N H_2SO_4 prior to microbiological assay for biotin. "Folic acid," biotin and pantothenic acid were deter-

[1] We are indebted to the Lederle Laboratories for a supply of crystalline Lactobacillus casei factor ("folic acid") used as a microbiological standard.

mined microbiologically in the liver samples by the same procedures used in the fecal assays.

RESULTS

The growth data on the animals of the various groups are summarized in figure 1. During the pre-sulfonamide period of feeding the

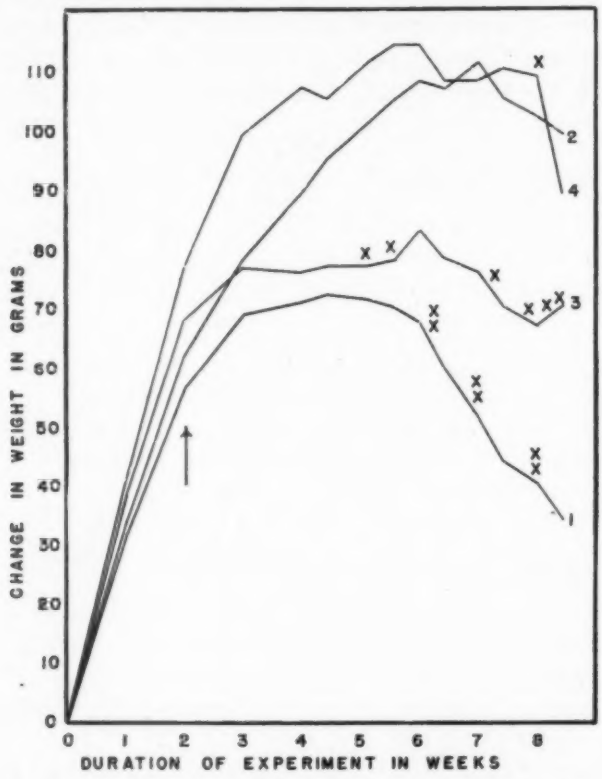

Fig. 1 Growth curves of rats receiving various diets: (1) High in carbohydrate, (2) high in protein, (3) high in fat, (4) high in protein and fat. The arrow indicates the inclusion of SST in the diets. An X indicates the death of one animal.

rats consuming the high fat diets grew at a more rapid rate, presumably as a result of an increased caloric intake per gram of diet consumed. When SST was included in the diet the animals consuming the high protein diets continued to gain weight and only ceased growing after consuming the 5% sulfonamide diet for 4 weeks. The animals were normal in appearance and free from evidences of deficiency until

they began to decline in weight. In contrast, when 5% SST was included in the high carbohydrate or high fat diets growth soon ceased and all animals became untidy in appearance. Alopecia, porphyrin-caked whiskers or ophthalmitis was seen in most of the animals of these groups. Deaths were frequent in the high carbohydrate or high fat groups whereas only one animal died in the groups ingesting the high protein diets.

The bacterial counts on the feces from animals of the various groups are shown in figure 2. SST was equally effective in each of the four groups in reducing the coliform count from an initial value of approximately 40 million organisms to approximately 200 thousand organisms

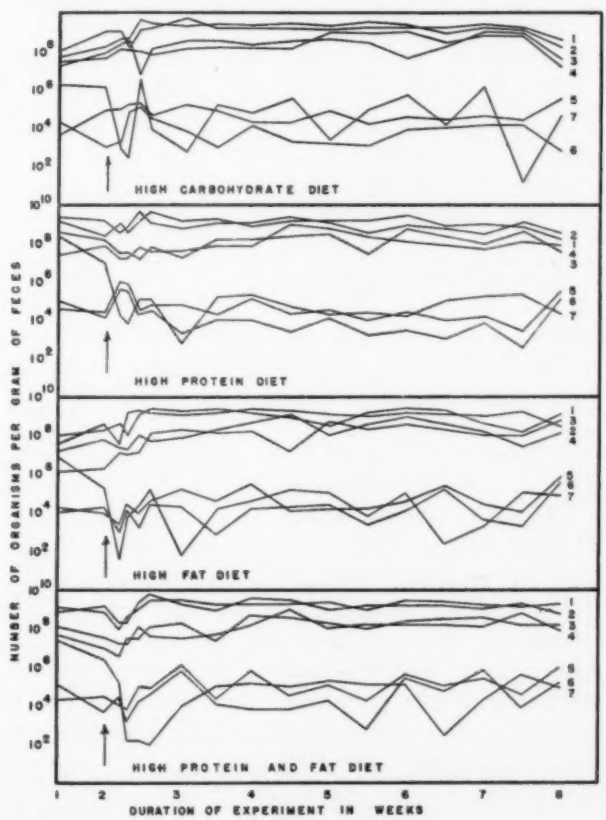
HIGH CARBOHYDRATE DIET
HIGH PROTEIN DIET
HIGH FAT DIET
HIGH PROTEIN AND FAT DIET
NUMBER OF ORGANISMS PER GRAM OF FECES
DURATION OF EXPERIMENT IN WEEKS

Fig. 2 Fecal elimination of bacteria as influenced by diet: (1) Total anaerobes, (2) total aerobes, (3) streptococci, (4) lactobacilli, (5) anaerobic spores, (6) aerobic spores, (7) coliforms. The arrow indicates the inclusion of SST in the diets.

per gram. The total number of organisms per gram of feces was unaffected by the inclusion of 5% SST in the various diets.

Prior to the inclusion of SST the fecal elimination of "folic acid," biotin and pantothenic acid was not affected by the composition of the diet. When SST was fed the fecal elimination of "folic acid," biotin and pantothenic acid was markedly reduced in all groups but the reduction was generally less when the diets were high in protein.

The results of occasional determinations of the liver storage of "folic acid," biotin and pantothenic acid are given in table 3. The inclusion of 5% SST in the highly purified diets was accompanied by a

TABLE 3

The liver storage of "folic acid," biotin, and pantothenic acid in individual rats receiving various diets.

TREATMENT	DURATION OF FEEDING	"FOLIC ACID"				BIOTIN				PANTOTHENIC ACID			
		I	II	III	IV	I	II	III	IV	I	II	III	IV
	days	*μg./gm.*				*μg./gm.*				*μg./gm.*			
No sulfonamide	15	4.5	10.5	3.8	3.9	...	...	...	...	...	...	...	...
5% SST	32	0.31	0.30	0.29	0.23	0.27	0.33	0.31	0.17	57	107	58	69
5% SST	43	0.21	0.32	0.18	0.31	0.22	0.21	0.20	0.17	54	100	63	88
5% SST	61 [1]	0.39	0.46	0.25	0.31	0.29	0.22	0.26	0.25	50	56	46	52

I = high carbohydrate diet; II = high protein diet; III = high fat diet; IV = high protein and fat diet.

[1] The results given are the average of data obtained on three rats.

decrease in the hepatic storage of these factors. The stores of "folic acid" in the liver tended to be higher in those animals consuming the high protein diets. There appeared to be no difference in the storage of biotin with respect to variations in the composition of the diet. The hepatic storage of pantothenic acid was considerably reduced below that usually found in stock animals in only those groups receiving the 18% casein diets. The animals receiving the high protein diets (30% casein) appeared to maintain a normal storage of pantothenic acid until they ultimately ceased growing. The levels of pantothenic acid eventually reached in all four groups were indicative of incipient pantothenic acid deficiency (Wright and Welch, '44).

DISCUSSION

The results obtained have shown that the composition of the diet is an important factor in the production of nutritional deficiencies by the

use of SST. Diets high in protein in some manner delayed the onset of the deficiency syndrome which is induced by the inclusion of SST in the usual highly purified diets. The superiority of the diets high in protein could not be correlated with changes in the number or types of organisms present in the intestine. This would indicate that if the mechanism by which poorly absorbed sulfonamides act has a bacteriological basis, it must depend on an alteration in the metabolism rather than on changes in the actual number of the intestinal organisms present. The observations of Miller ('44) are consistent with these findings.

It seems difficult, in view of the small and sometimes inconsistent differences observed in the fecal elimination of "folic acid," biotin and pantothenic acid within the various dietary groups, to ascribe the superiority of the high protein diets solely to an increased intestinal synthesis of these factors. The hepatic storage of "folic acid" likewise, was not appreciably greater in the animals consuming high protein diets and seems insufficient to explain the growth results obtained. In the case of pantothenic acid, however, the hepatic storage in rats receiving high protein diets remained at normal levels until the rats had begun to decline in weight. Possibly the greater storage of pantothenic acid was a factor in explaining the superiority of the high protein diets.

There is much evidence that pantothenic acid is involved in carbohydrate metabolism (Williams, '43) and the requirement for this factor might reasonably be expected to be lower on a high protein than on a high carbohydrate or high fat diet. The apparent superiority of the high protein diets may actually be due to the fact that in increasing the protein content of the diet the carbohydrate has been correspondingly reduced.

These experiments have been limited to a consideration of the deficiencies of "folic acid," biotin and pantothenic acid. It is quite possible that the level of SST employed in these studies is capable of inducing additional deficiencies which are directly influenced by the level of dietary carbohydrate, protein or fat.

SUMMARY

1. A study has been made of the growth, the bacterial flora of the feces, and the fecal elimination and liver storage of "folic acid," biotin and pantothenic acid in rats fed succinylsulfathiazole (SST) containing diets high in either carbohydrate, protein, fat, or both protein and fat.

2. Weight gains continued for a longer period of time in rats fed SST in high protein diets than in animals receiving the drug in high carbohydrate or high fat diets. Some of the signs of nutritional deficiencies which early became apparent in the latter groups did not appear in animals fed the high protein diets.

3. The bacterial flora of the feces was not demonstrably altered in number or kind of organisms by changes in the composition of the diet.

4. The superior condition of the rats ingesting high protein diets showed only slight correlation with the fecal elimination of "folic acid," biotin and pantothenic acid.

5. The inclusion of SST in all the diets was accompanied by a marked reduction in the hepatic stores of "folic acid" and biotin. The stores of "folic acid" were, however, somewhat higher in the rats receiving the high protein diets.

6. The hepatic stores of pantothenic acid remained high in the animals receiving the high protein diets until they eventually began to lose weight. Of the determinations made, the hepatic stores of pantothenic acid showed the best correlation with the condition of the animals.

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